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# JOURNAL OF AGRICULTURAL RESEARCH

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# JOURNAL OF AGRICULTURAL RESEARCH

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No. 14

## CALIFORNIA GREEN LACEWING FLY

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### INTRODUCTION

The green lacewing fly (*Chrysopa californica* Coquillett) (fig. 1) has been observed by the writer on many occasions during the past five years in connection with outbreaks of aphids in southern Arizona and California, and at various times the extreme usefulness of the species in controlling these outbreaks has been noted. An opportunity for making

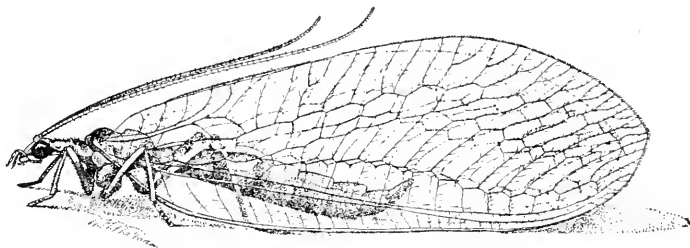


FIG. 1.—The California green lacewing fly (*Chrysopa californica*): Adult.

a complete study of the species came to hand during the past year (1915), and this paper is prepared for the purpose of recording the facts as observed and interpreted during this study.

### HISTORICAL REVIEW

This lacewing fly has been known since 1890, when it was collected in California and described by Coquillett (3)<sup>1</sup> in the Report of the State Board of Horticulture of that State. The next reference (1, p. 156) to it is in Banks's Revision of the Nearctic Chrysopidae. In this paper, published in 1903, Mr. Banks redescribed the species and stated that "it is the most abundant species on the Pacific coast."

It was not, however, until the year 1912 that the real usefulness and economic value of this species was brought forth. In this year

<sup>1</sup> Reference is made by number to "Literature cited," p. 524.

Quayle (7) listed it as an enemy of the Citrus red spider (*Tetranychus mytilaspidis* Riley) and remarked that "this is the commonest of the predatory insects occurring on citrus trees." Two years later the species was mentioned as an enemy of the Citrus red spider by Ewing (6). Essig (4), in 1911, described it briefly and included a few remarks on its habits and hosts. In 1913 (5), in his "California Insects," he credited this species of *Chrysopa* with feeding upon 14 different species of insects.

In August (2), 1915, and again in October (2), Mr. E. J. Branigan, a deputy of the California Horticultural Commission, referred to the economic importance of this species. In the first citation he reported the insect as feeding upon the "elm-leaf cluster louse." He stated: "Large numbers of the egg clusters of *Chrysopa californica* were present, the larvæ upon hatching, burrowing into the leaf clusters and feeding upon the lice." In October he reported this lacewing larva as attacking a citrus mealy bug (*Pseudococcus* sp.), and states further: "The green lacewing was found to be heavily parasitized by several species of parasites."

#### DISTRIBUTION OF THE FLY

From our present knowledge of the species it is distinctly of western distribution, occurring throughout the Pacific Coast States, Texas, Arizona, New Mexico, Nevada, Lower California, and doubtless Utah. As early as 1903, Banks (1) stated:

I have seen specimens from many places; Los Angeles, Tehama, Wanona [Wawona?], San Bernardino, Palo Alto, San Mateo County, Santa Clara County and Siskiyou [Siskiyou?] County, mostly in July and August, but some in April; also from Hood River, Oregon, September; Pullman, Wash., July and August; and King's Canon, Ormsby County, Nevada, July.

Mr. C. N. Ainslie, of the Bureau of Entomology, has taken a specimen of *Chrysopa* sp. at Salt Lake City, Utah, which is without much doubt this species. The writer has taken or seen specimens in southern California, Lower California, Mexico, Arizona, New Mexico, and in many different localities in these States at elevations varying from sea level to 7,000 feet

#### HOST INSECTS

While the larvæ of this lacewing fly, as well as Chrysopidae in general, feed primarily upon aphids, their good work is far from being restricted to this group of insects. Mites, leafhoppers, thrips, and doubtless many other insects sufficiently small to be easily captured and devoured are likewise eaten.

Essig (5) has shown the following 14 species of insects to be attacked by *Chrysopa californica*:

- Clover mite (*Bryobia pratensis* Garman).
- Two-spotted mite (*Tetranychus mytilaspidis* Riley).
- Red spider (*T. telarius* Linnaeus).
- Apple-leafhopper (*Empoasca mali* Le Baron).

Grape leafhopper (*Typhlocyba comes* Say).  
 The pear Psylla (*Psylla pyricola* Foerster).  
 Mealy plum plant louse (*Hyalopterus arundinis* Fabricius).  
 Melon aphid (*Aphis gossypii* Glover).  
 Black peach aphid (*Aphis persicæ-niger* Erwin Smith).  
 Green Citrus plant louse (*Macrosiphum citrifolii* Ashmead).  
 Citrus mealy bug (*Pseudococcus citri* Risso).  
 Frosted scale (*Eulecanium prunosum* Coquillett).  
 Red scale (*Chrysomphalus aurantii* Maskell).  
 Purple scale (*Lepidosaphes beckii* Newman).

Mr. E. G. Smyth, working at Tempe, Ariz., found larvæ of *C. californica* feeding also on the wheat thrips (*Euthrips tritici* Fitch), which they apparently preferred to the pea aphid (*Macrosiphum pisi* Kaltendach). Mr. R. N. Wilson, also at the Tempe laboratory, observed larvæ of *C. californica* feeding upon the barley mite (*Notophallus viridis* Banks) and on the "green bug" (*Toxoptera graminum* Rondani), while the writer reared the species exclusively on the corn leaf aphid (*Aphis maidis* Fitch), it being a very important check upon this pest.

#### LIFE HISTORY AND HABITS OF THE LACEWING FLY

##### THE ADULT

As before stated, this species of *Chrysopa* was first described by Coquillett (3) in 1890, and later (in 1903) redescribed by Banks (1). The original description of the adult by Coquillett is as follows:

Pale green, a yellowish white dorsal stripe extends from front of thorax to tip of abdomen; front of head whitish; an irregular wine-red stripe extends from each eye to the mouth, and on its hind border, next the eye, is a black streak; front corners of thorax marked with black. Antennæ pale yellowish, minutely ringed with white. Wings greenish hyaline, obtusely pointed at their tips; veins and veinlets wholly green; seven or eight of the veinlets along the hind edge of front wings before the tips are forked; stigma somewhat opaque, yellowish green; legs green, tarsi whitish, the tips brown. Eyes greenish golden, becoming glaucous brown after death. In dried specimens the green coloring becomes more yellowish and the tarsi assumes a slightly darker color than the tibiae. Length 9 to 10 mm. (about  $\frac{3}{8}$  of an inch); expands from 24 to 28 mm. (about one inch or slightly over).

The adults are delicate green, flitting creatures which dart up from the shady protecting vegetation as one walks along a fence row or through an alfalfa field. The males are slightly smaller than the females and appear more vivid in color. During the breeding season both are short-lived. Neither sex has ever been noted by the writer to feed in the adult stage, even when food was offered, and doubtless all of the lacewing flies take little or no food in this period of their existence.

Copulation takes place almost immediately after the adults have issued and become dry, and in all cases under observation the male was dead on the following day. Oviposition usually begins the day following copulation and may continue for a period of three or four days, or the full complement of eggs may be deposited in a single day. Four

females under observation (see Table I) laid an average of  $30\frac{3}{4}$  eggs each, the record being 34, 25, 38, and 26 eggs, respectively. The females, after performing what is apparently their sole purpose in life, die within 24 to 36 hours after oviposition is completed. The adults are especially numerous in southern Arizona during February, March, April, and May, and again during October and November.

## THE EGG

The egg (fig. 2) is placed on a long stalk or pedicel, which is hair-like and about half an inch in length. The egg itself is oblong and very small; at first it is whitish, but in a day or two it darkens and thereafter until it hatches the segmentation of the developing larva is revealed through the eggshell. It has a button or lid at the upper end, which is slightly flattened, while the lower end tapers until it is barely larger than the stalk to which it is attached. The original description by Coquillett (3) is as follows:



FIG. 2.—*Chrysopea californica*: Eggs.

Very pale blue, elongate-ovate, pointed at the base, the apex flattened and in its center is a white button-shaped object; surface minutely granulated; length, three and one-half hundredths of an inch; mounted on a bristle-like pedicel from thirteen to eighteen hundredths of an inch long.

The egg stage (see Table I) was found to vary, being from 6 to 12 days in duration under the temperatures at which the experiment was carried on. The average time required for the 122 eggs under observation was 8 days.

TABLE I.—The egg stage of *Chrysopea californica* at Tempe, Ariz., in 1915

Female No.	Cage No.	Eggs.				Length of stage.	Average mean temperature.
		Date laid.	Number.	Date hatched.	Number.		
1.....	T 78.....	Feb. 11	11	Feb. 23	11	Days.	° F.
	T 79.....	Feb. 12	7	{ ..do....	1	12	53
	T 80.....	Feb. 13	15	{ ..do....	6	11	53
				{ Feb. 25	5	12	53
2.....	T 81.....	Feb. 14	1	{ ..do....	1	11	53
	T 1159.....	Oct. 12	19	Oct. 19	19	7	66.5
	T 1160.....	Oct. 13	6	Oct. 20	6	7	67
3.....	T 1185.....	..do....	20	{ ..do....	20	7	67
	T 1186.....	Oct. 14	13	{ ..do....	13	6	68
	T 1187.....	Oct. 15	4	Oct. 21	4	6	69
4.....	T 1189.....	Oct. 16	1	Oct. 23	1	7	70
	T 1190.....	..do....	15	Oct. 22	14	6	70
	T 1196.....	Oct. 17	11	Oct. 23	11	6	70.5
Total or average			123		122	8	.....

## THE LARVA

The larva when first hatched (fig. 3) is a delicate, white, nearly colorless object, quite conspicuously hairy and with mandibles which are large in comparison to the size of the body, these being about one-fourth its entire length. Coquillett's (3) description follows:

Mixed with a yellowish white and pinkish brown, the latter color forming a dorsal line and a series of lateral spots; along each side of the body is a row of yellowish white tubercles; head yellowish white, marked with two diverging black stripes on the top, and with a dusky streak each side, having in its middle a black dot; length, 7 mm. (A little over one-fourth of an inch.)

## LARVAL HABITS

The hatching process requires but a few minutes, but the larva rests on the empty eggshell for some time after emergence. When the eggshell becomes dry and hardened, the larva hastily crawls down the supporting egg stalk and eagerly begins searching for food. If small aphids or thrips nymphs are present, it quickly seizes one of these and begins feeding. If only full-grown and large aphids are present, it is more cautious, running in a circle around the tempting and monstrous meal or following the aphid, ever and anon stopping as if to consider whether or not it could safely attack a creature so many times larger than itself. Finally, however, its increasing hunger apparently overcomes all fear and it pounces on its prey. The aphid is lifted bodily off its feet, the lacewing larva all the time crushing, piercing, and sucking its prey. The larvæ of all lacewing flies extract their food from the host by piercing it with their long, powerful mandibles, which are hollow, the internal fluids of the host being rapidly absorbed through them. With abundant food present the larva grows rapidly and quickly takes on a robust appearance.



FIG. 3.—*Chrysopa californica*: First instar.

## LARVAL DEVELOPMENT

The larvæ in the course of their development molt twice, which divides the larval period into three instars, with a total length of from 11 to 22 days, depending upon the prevailing temperature, the average length being about 16 days. (See Table II.) During this period from 74 to 160 full-grown aphids were eaten by each larva, the number consumed depending upon the temperature, the larvæ being more active and voracious during warmer weather.

TABLE II.—Table of molts and instars of *Chrysopa californica* at Tempe, Ariz.

PART I. FEBRUARY, 1915. AVERAGE MEAN TEMPERATURE, 54° F.

Gage No.	Date hatched.	Date of first molt.	Length of first instar.	Number of aphids eaten.	Date of second molt.	Length of second instar.	Number of aphids eaten.	Date in cocoon.	Length of third instar.	Number of aphids eaten.	Total number of aphids eaten.	Total length of larval period.
			Days.			Days.			Days.			Days.
78-1.....	Feb. 23	Mar. 3	8	15	Mar. 10	7	10	Mar. 17	7	43	68	22
78-2.....	do	Mar. 2	7	15	Mar. 9	7	15	do	8	59	89	22
78-3.....	do	Mar. 3	8	17	do	6	12	do	8	61	90	22
78-4.....	do	do	8	17	Mar. 11	8	16	do	6	50	83	22
79-1.....	Feb. 24	Mar. 4	8	12	do	7	16	do	6	47	75	21
79-2.....	do	do	8	10	do	7	16	do	6	51	77	21
79-5.....	do	Mar. 5	9	12	Mar. 12	7	23	do	5	39	74	21
80-3.....	Feb. 25	do	8	12	do	7	22	do	5	49	83	20
80-5.....	do	do	8	11	do	7	19	Mar. 18	6	74	104	21
80-6.....	do	do	8	10	do	7	15	Mar. 17	5	56	81	20
80-7.....	do	do	8	13	Mar. 11	6	25	do	6	62	100	20
80-9.....	do	do	8	14	Mar. 12	7	27	do	5	56	97	20
80-10.....	do	do	8	14	Mar. 11	6	28	do	6	63	105	20
80-11.....	do	do	8	13	Mar. 12	7	20	do	5	56	89	20
80-12.....	do	do	8	15	Mar. 15	10	32	Mar. 19	.....	57	104	.....
Average.....			8	13½		7	20		6	55½	88	20¾

PART II. OCTOBER, 1915. AVERAGE MEAN TEMPERATURE, 70° F.

59-2.....	Oct. 19	Oct. 23	4	14	Oct. 26	3	21	Oct. 30	4	108	143	11
59-3.....	do	do	4	17	Oct. 27	4	33	Nov. 1	5	70	120	13
59-4.....	do	do	4	16	Oct. 20	3	18	do	6	109	143	13
59-6.....	do	do	4	15	do	3	34	Oct. 31	5	100	147	12
59-7.....	do	do	4	13	Oct. 27	4	35	do	4	95	143	12
59-9.....	do	do	4	13	do	4	35	do	4	90	139	12
59-10.....	do	do	4	15	do	4	26	do	4	93	134	12
59-11.....	do	do	4	16	Oct. 26	3	30	do	5	114	160	12
59-12.....	do	do	4	14	Oct. 27	4	36	do	4	91	141	12
59-13.....	do	do	4	14	do	4	32	do	4	100	146	12
59-14.....	do	do	4	12	do	4	39	do	4	107	158	12
Average.....			4	14½		3½	31		4½	98	143	12

<sup>1</sup> Died.

From these records it is seen that a *C. californica* larva under natural conditions, eating both large and small aphids, must often consume 300 or 400 of them during the course of its development. The economic value of these larvæ is thus seen to be enormous. It was found that an average of about 14 full-grown adults of *Aphis maidis* were consumed in the first instar, 4 to 7 aphids being eaten the first day after hatching. The duration of the first instar was found to vary with the temperature, it being from 4 to 9 days and the average period about 6 days in length. A great many more aphids are consumed during the second instar than in the first. This instar averages nearly a day shorter, being 7 days during March and 3¾ days during October, 20 being the average number of aphids eaten by each of 15 larvæ during the former period and 31 during the latter. In actions and habits it is largely the same as the first instar except for the increased power of destroying aphids

The third-instar larvæ (fig. 4), while having a period of life averaging about the same in length as that of the second instar, make up for it in the number of aphids consumed. Fifteen larvæ in March each ate an average of 9½ aphids a day or 55½ during the entire period; whereas 11 larvæ each ate an average of nearly 22 full-grown aphids a day or 98 for the third-instar period, this being nearly twice as many as are eaten during the first and second instars. In Table IV it will be noted that a third-instar larva of *C. californica* in cage 59-14 ate 40 full-grown *Aphis maidis* in one day of 24 hours. The average length of the third instar was 6 days in March and 4½ days in October.

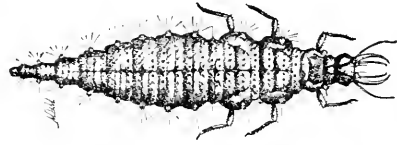


FIG. 4.—*Chrysopa californica*: Third instar.

Tables III and IV show the daily consumption of aphids by 26 larvæ during their entire larval period.

TABLE III.—Daily feeding record of 15 larvæ of *Chrysopa californica* at Tempe, Ariz., in February, 1915<sup>a</sup>

Cage No.	Date hatched.	February.					March.					
		24	25	26	27	28	1	2	3	4	5	6
78-1	Feb. 23	5	5	2	2	0	0	1	m 2	3	3	0
78-2	do	4	4	4	1	2	0	m 0	5	5	5	0
78-3	do	5	3	5	2	0	0	2	m 3	4	5	0
78-5	do	4	5	2	3	0	1	2	m 0	3	5	2
79-1	Feb. 24	2	4	2	2	2	0	0	2	m 1	3	5
79-2	do	2	2	3	1	1	0	0	1	m 0	4	8
79-5	do	4	3	0	3	2	0	0	0	0	m 5	5
80-3	Feb. 25	3	4	1	3	1	0	0	0	0	m 3	4
80-5	do	3	1	4	2	3	0	0	0	1	m 0	7
80-6	do	2	2	2	2	3	0	0	1	0	m 4	3
80-7	do	3	2	3	4	0	1	0	1	0	m 4	8
80-9	do	4	3	1	4	1	0	1	0	1	m 2	7
80-10	do	4	3	1	4	2	0	0	0	0	m 5	7
80-11	do	4	2	0	5	2	0	0	0	0	m 4	6
80-12	do	4	5	0	3	3	0	0	0	0	m 3	3

Cage No.	Date hatched.	March.																			Average mean temperature.
		7	8	9	10	11	12	13	14	15	16	17	18	19	20						
78-1	Feb. 23	2	0	0	m 3	7	7	8	7	7	4	c								°F.	
78-2	do	0	0	m 5	10	10	10	8	4	2	c									54	
78-3	do	0	0	m 3	9	10	10	10	4	5	c									54	
78-5	do	5	1	0	0	m 5	10	9	8	10	c									54	
79-1	Feb. 24	6	1	0	0	m 3	9	9	8	9	c									54	
79-2	do	3	1	0	0	m 8	9	10	10	4	c									54	
79-5	do	6	6	0	0	1	m 4	10	10	8	7	c								54	
80-3	Feb. 25	5	7	3	0	0	m 3	10	12	10	14	c								54	
80-5	do	0	8	2	2	0	m 6	10	13	15	15	15	c							54	
80-6	do	5	3	0	0	0	m 8	8	12	14	14	c								54	
80-7	do	7	6	0	0	m 0	10	10	13	15	14	c								54	
80-9	do	6	8	3	1	0	m 8	9	12	12	15	c								54	
80-10	do	8	7	0	1	m 0	8	10	15	15	15	c								54	
80-11	do	2	7	1	0	0	m 6	8	12	15	15	c								54	
80-12	do	7	5	0	4	8	2	0	0	m 8	12	12	12	13	c					54	

<sup>a</sup> m, Date of molting; c, date of spinning cocoon.

TABLE IV.—Daily feeding record of 11 larvæ of *Chrysopa californica* at Tempe, Ariz., in October, 1915<sup>a</sup>

Care No.	Date hatched.	October.													November.	Average mean temperature.	
		20	21	22	23	24	25	26	27	28	29	30	31	1			
59-2.....	Oct. 19.....	7	7	0	m 6	15	0	m 18	20	25	25	c 20	.....	.....	.....	°F.	70
59-3.....	do.....	7	7	3	m 7	14	12	0	m 16	23	28	3	0	c	.....	.....	70
59-4.....	do.....	5	7	4	m 7	11	0	m 17	19	22	26	25	0	c	.....	.....	70
59-6.....	do.....	7	7	1	m 7	15	12	m 10	18	25	25	22	c	.....	.....	.....	70
59-7.....	do.....	6	7	0	m 7	14	14	0	m 20	24	23	28	c	.....	.....	.....	70
59-9.....	do.....	6	5	2	m 7	14	14	0	m 10	25	25	30	c	.....	.....	.....	70
59-10.....	do.....	7	6	2	m 6	15	5	0	m 19	24	26	24	c	.....	.....	.....	70
59-11.....	do.....	7	7	2	m 7	15	8	m 14	20	25	25	30	c	.....	.....	.....	70
59-12.....	do.....	7	7	0	m 7	15	14	0	m 20	25	23	23	c	.....	.....	.....	70
59-13.....	do.....	5	7	2	m 6	15	11	0	m 20	25	25	30	c	.....	.....	.....	70
59-14.....	do.....	6	6	0	m 7	15	17	0	m 19	23	25	40	c	.....	.....	.....	70

<sup>a</sup> m, Date of molting; c, date of spinning cocoon.

Table III shows the record of 15 larvæ during the month of February and Table IV shows the record of 11 larvæ during the month of October. It will be noted that both the daily and total consumption were much larger during the latter and warmer period than during the former and that the total feeding period was nearly half the length during this period. Only full-grown wingless specimens of *Aphis maidis* were used in this experiment.



FIG. 5.—*Chrysopa californica*: Pupal case.

MOLTING

When the larva gets ready to molt, it settles down in some protected spot and rests for a period of several hours, often a day or more, and when the opportune time seems to have arrived it begins a series of movements, mostly of a rising and falling nature, calculated to burst the skin on the back. When this is finally accomplished, it crawls out and, after a few minutes' rest, is the same voracious creature it was before except only that its size is greater than in the preceding instar.

During the first and second instars, after the larva has eaten its quota of aphids, it rests, often as long as two days; during the last instar, however, this rest period is not apparent, owing to the fact that it takes place within the cocoon previous to pupation.

As shown by dissections of several cocoons, this resting period, during which the pupa is forming within the larval skin, is from 6 to 9 days in length. Later in the observations it was discovered that one could tell by external indications just when this change

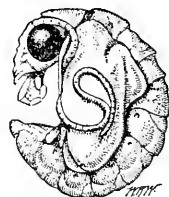


FIG. 6.—*Chrysopa californica*: Pupa.



takes place. The larval skin when shed by the pupa is circular in form and is pressed firmly against one end of the pupal case, appearing from without and through the wall of the cocoon (fig. 5) as a dark, almost black, disk.

THE PUPA

The pupa (fig. 6) is formed within a membranous case or cocoon (fig. 5, 7) which is nearly globular in shape, tough but pliable, and inclosed or surrounded by numerous white filaments which hold it in place on the leaf or in some protecting cavity. The cases are often found singly, but when the infestation has been heavy, they may be in groups of a dozen or more. Mr. L. J. Hogg, an assistant, found as many as a dozen or more in a single curled ash leaf, the larvæ having fed on the elm-leaf cluster aphid.

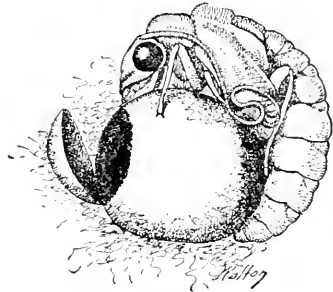


FIG. 7.—*Chrysopa californica*: Pupa freshly emerged from its cocoon.

TABLE V.—Length of the pupal stage of *Chrysopa californica* at Tempe, Ariz., in 1915

Cage No.	Date of pupation.	Date adult issued.	Stage length.	Average mean temperature.
			Days.	°F.
78-2	Mar. 17	Apr. 1	15	63
78-3	do.	do.	15	63
78-5	do.	Apr. 2	16	63
79-1	do.	Apr. 4	18	63
79-5	do.	Apr. 7	21	63
80-3	do.	Apr. 2	16	63
80-5	Mar. 18	Apr. 5	18	63
80-6	Mar. 17	Apr. 1	15	63
80-7	do.	do.	15	63
80-10	do.	Mar. 31	14	63
80-11	do.	Apr. 3	17	63
59-2	Oct. 30	Nov. 19	19	57
59-3	Nov. 1	Nov. 20	20	57
59-4	do.	Nov. 21	21	57
59-6	Oct. 31	Nov. 20	20	57
59-7	do.	do.	20	57
59-9	do.	Nov. 21	21	57
59-10	do.	Nov. 19	19	57
59-11	do.	Nov. 22	22	57
59-12	do.	Nov. 19	19	57
59-13	do.	Nov. 22	22	57
59-14	do.	Nov. 20	20	57
Average			18	

The pupal stage in southern Arizona (see Table V) varied from 14 days to 23 days in length, the average being 16 1/8 days for March and 20 7/11 days for November.

As has been mentioned, the larva, after constructing the pupal case, which often requires a day's time, may remain several days before pupating. The pupa when formed is curled up as shown in figure 6, with the abdomen closely folded between the large thick wing pads. When ready to change to an adult, the pupa emerges from the cocoon (see fig. 7) through a circular lid, and in from  $\frac{1}{2}$  hour to 2 hours the pupal skin is shed and the adult (fig. 1) comes forth. After a few minutes have been allowed for the expansion and drying of the wings, the lacewing fly is ready for flight.

#### SEASONAL HISTORY AND HIBERNATION

From the writer's observations during the past year (1915) in the Salt River Valley of Arizona, there are at least six generations annually. The first covers the period from about February 15 to March 15, and the remaining generations follow one another every 40 to 45 days from then until late October, either the pupa or adults of the last generation going into hibernation at that time. Adults can be taken throughout the winter months, but eggs have never been secured until the advent of milder weather. Pupæ are often taken during any of the winter months in the Salt River Valley of Arizona, which has a mild winter climate.

#### NATURAL ENEMIES OF THE LACEWING FLY

It seems that in California (2) the species is commonly attacked by several species of parasites, but no record of any parasite has been obtained during the present study, although abundant material of this lacewing fly was examined. Robber flies have been noted to catch the adults, and certain Hemiptera prey upon the larvæ, but with these exceptions this lacewing fly seems to be quite free and unmolested.<sup>1</sup>

According to the records of the Biological Survey, United States Department of Agriculture, the Western wood pewee (*Contopus richardsonii*) feeds upon the species at Pasadena, Cal.; and at East Bernard, Tex., the nighthawk (*Chordeiles virginianus*) was found feeding upon the species, the stomachs of two birds containing three and six adults, respectively.

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<sup>1</sup> Possibly this is due to the extremely offensive odor thrown off by the adults of all lacewing flies when alarmed.

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## RAPE AS MATERIAL FOR SILAGE

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### INTRODUCTION

The popularity of rape (*Brassica napus*) as a pasture crop has been steadily increasing since its introduction into this country about 25 years ago. Its value as such is considerable, but its usefulness would be greatly increased if it could be preserved in the silo and used successfully as a succulent feed for the winter months. Attempts to ensile it have, however, evidently been few, perhaps since it has generally been considered too watery for this purpose. The only report of such an attempt which has been found in the literature is from Canada.<sup>1</sup>

In that experiment rape was cut when about 15 inches high and ensiled alone and with an equal weight of corn. When the silage was fed six months later, it was said to have been well preserved, to have had a pleasant odor, and to have been eaten with avidity by cattle. Chemical analyses showed a considerable loss of water and carbohydrates and an increase in nonprotein nitrogen. With the exception of the loss of water, these losses are not much greater than the losses which occur in ensiling the corn plant (*Zea mays*). In that experiment the total loss of dry matter was 26.5 per cent. Weight for weight, however, rape silage was found by analysis to be a much more valuable feeding material than green rape.

In 1914, Evvard, at the Iowa Station,<sup>2</sup> made rape silage in barrels, with and without the addition of common salt (sodium chlorid). The highly salted silage was quite well preserved and had a favorable odor, but was refused by stock. The unsalted silage contained mold and had undergone some putrefactive fermentation, the odor of volatile sulphids being quite evident. The shape of the barrels and the consequent difficulty of excluding air on the settling of the ensiled material were responsible for this putrefaction. This emphasizes the importance of using suitable air-tight containers in making rape silage.

The ideal plant for silage making must contain just sufficient fermentable sugars to furnish enough acids to preserve it. In most respects the corn plant furnishes the most nearly ideal material for silage. The legumes are not ensiled so successfully because the percentage of protein is too high for the amount of sugar, and some putrefaction is likely to

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<sup>1</sup> Schutt, F. T. Report of the chemist. Fodders and feeding stuffs. *In Canada Exp. Farms Rpts.* 1904, p. 166-182. 1905.

<sup>2</sup> Unpublished data.

occur. Rape contains a larger amount of sugars<sup>1</sup> and is therefore likely to develop a high acidity. Rape, in common with other Cruciferae, contains considerable amounts of organic sulphur compounds, which are likely to form disagreeable volatile products if the fermentation progresses too far. For these reasons a mixture of rape and a legume should produce better silage than either alone.

#### EXPERIMENTAL RAPE SILAGE

The experimental silage was therefore made from rape alone and from mixtures of rape with various other materials, as outlined in Table I, with the purpose of determining the most satisfactory combination. The other plant materials used were alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), sweet clover (*Melilotus alba*), potato tubers (*Solanum tuberosum*), timothy (*Phleum pratense*), Sudan grass (*Andropogon sorghum, aethiopicus*), sorghum cane (*Sorghum vulgare*), and bluegrass (*Poa pratensis*). The rape used was quite mature but still succulent. The rape leaves were cut off at the main stalk. The entire plant was cut 3 inches from the ground. The alfalfa was cut just before blooming. The corn, Sudan grass, and sorghum cane used were mature. The other plant materials were cut just before maturity. All the forage was cut by a silage cutter into half-inch lengths. The material was tightly packed into glass jars of about 1-gallon capacity, in the same manner as corn silage has repeatedly been made in this laboratory. The jars were closed with metal caps, which were not too tight to prevent the escape of excess gases.

<sup>1</sup> An average air-dry sample contains 5.00 per cent of total fermentable sugars calculated as dextrose.

TABLE I.—Analyses of rape silage

Sample No.	Percentage of constituents of sample.	Result of inspection.			Data on 100 c. of juice.										Calculated to 100 gm. of dry matter.				
		Appearance.	Odor.	Taste.	Class.	Water.	Total acidity.		Volatile acidity.		Fixed acidity (by difference) lactic acid.	Alcohol.	Amino nitrogen.	Total acidity calculated as lactic acid.	Volatile acidity calculated as acetic acid.	Fixed acidity (by difference) lactic acid.	Alcohol.	Amino nitrogen.	
							N/10 solution.	Gm. as lactic acid.	N/10 solution.	Gm. as acetic acid.									Gm.
1	Rape entire.....	Good.....	Pleasant.....	Sour.....	B	Per cent.	C. c.	Gm.	C. c.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
2	Rape leaves.....	do.....	do.....	Very sour.....	C	84.5	275	2.475	0.401	1.079	0.016	0.281	13.572	2.827	12.243	0.220	1.724		
3	Rape 80, alfalfa 20	do.....	Aromatic.....	do.....	B	86.0	298	2.682	76.8	0.401	0.016	0.281	16.152	2.827	12.243	0.220	1.724		
4	Rape 60, alfalfa 40	do.....	Unpleasant.....	Disagreeable <sup>b</sup> .....	E	84.7	252	2.268	1.791	1.093	0.016	0.281	12.538	2.827	12.243	0.220	1.724		
5	Rape 40, alfalfa 60	do.....	Aromatic.....	Good, but sour.....	A	83.1	189	1.701	1.093	1.093	0.016	0.281	8.503	2.827	12.243	0.220	1.724		
6	Rape leaves 80, alfalfa 20.	do.....	Sauerkraut.....	Very sour.....	C	85.1	227	2.043	128.2	7.69	0.000	0.268	10.063	4.634	6.606	0.361	1.614		
7	Rape leaves 60, alfalfa 40.	do.....	do.....	Good, but sour.....	B	85.5	232	2.088	.....	.....	.....	.....	12.276	.....	.....	.....	.....		
8	Rape leaves 40, alfalfa 60.	do.....	Aromatic.....	Favorable.....	A	82.2	269	2.421	.....	.....	.....	.....	11.151	.....	.....	.....	.....		
9	Rape leaves 90, cane sugar 10.	do.....	do.....	Pleasant, but very sour.....	C	81.8	342	3.078	69.2	4.15	63.086	0.280	13.860	1.870	11.052	117.951	1.252		
10	Rape leaves 90, molasses 10.	Dark color.....	Agreeable.....	Too sour.....	C	82.3	301	2.709	48.8	2.203	62.041	.....	14.562	1.574	12.204	10.812	.....		
11	Rape 90, molasses 10.	do.....	do.....	do.....	C	82.1	351	3.159	.....	.....	.....	.....	14.490	.....	.....	.....	.....		
12	Rape leaves 80, molasses 20.	Dark.....	Like molasses.....	Sour.....	C	82.1	345	3.105	.....	.....	.....	.....	14.238	.....	.....	.....	.....		
13	Rape 80, starch 20	do.....	Starchy (slimy).....	do.....	D	75.4	234	2.106	.....	.....	.....	.....	6.453	.....	.....	.....	.....		
14	Rape leaves 80, starch 20.	do.....	do.....	Less sour.....	D	77.2	123	1.107	.....	.....	.....	.....	3.744	.....	.....	.....	.....		
15	Rape leaves 60, potatoes 40.	Good.....	Sour.....	Not bad.....	C	84.7	188	1.692	.....	.....	.....	.....	9.342	.....	.....	.....	.....		
16	Rape leaves 80, corn grain 20.	do.....	Good, but sour.....	Very sour.....	C-D	69.9	355	3.195	.....	.....	.....	.....	7.407	.....	.....	.....	.....		
17	Rape leaves 60, whole corn	do.....	Pleasant.....	do.....	C	79.4	342	3.078	.....	.....	.....	.....	11.880	.....	.....	.....	.....		
18	Plant 40, rape 60, corn grain 40.	do.....	Agreeable.....	Good, but sour.....	B	58.3	412	3.978	90.5	5.43	0.424	0.460	5.562	0.760	4.419	0.593	0.643		

<sup>a</sup> By aeration.

<sup>b</sup> Possibly accidental contamination.

<sup>c</sup> By distillation.

TABLE I.—Analyses of rape silage—Continued

Sample No.	Percentage of constituents of sample.	Result of inspection.			Water.	Data on 100 c. c. of juice.						Calculated to 100 gm. of dry matter.					
		Appearance.	Odor.	Taste.		Class.	Vol. acid.	W/sol.	Cal.	Fixed.	Alcohol.	Amino.	Vol.	Total.	Fixed.	Alcohol.	Amino.
						tion.	acid.	as lactic	tion.	acid.	as lactic	acid.	acid.	acid.	acid.	acid.	acid.
19	Rape leaves+100 gm. of calcium carbonate.	Dark.....	Disagreeable.....	"Rotten".....	F.....	81	0.759										
20	Rape+water.....	do.....	Strong.....	Disagreeable.....	E.....	131	1.179										
21	Rape leaves + water.	do.....	do.....	Disagreeable + sour.	E.....	155	1.395										
22	Rape leaves 60, red clover 40.	Good.....	Aromatic and pleasant.	Very palatable.....	B.....	272	2.448	73.6	3.432	1.752	b o. 147	6.224	12.114	2.186	8.838	0.727	1.109
23	Rape leaves 60, sweet clover 40.	do.....	Good (suggests sweet clover).	Bitter, sour.....	C.....	272	2.448						12.951				
24	Rape leaves+lactic-acid culture.	Dark.....	Rather disagreeable.	Unpleasant, biting.	D.....	222	1.998	70.9	4.25	1.359			12.504	2.075	8.550		
25	Rape leaves 60, blue grass 40.	Good.....	Good.....	Agreeable, but sour.	C.....	321	2.889						9.819				
26	Rape leaves 60, timothy 40.	do.....	Aromatic.....	Sour.....	C.....	341	3.069						14.472				
27	Rape 60, Sudan grass 40.	do.....	Aromatic like corn silage.	Slightly acid.....	B.....	330	2.970						8.478				
28	Rape leaves 60, sorghum cane 40.	do.....	do.....	Sour.....	C.....	329	2.961						8.604				

a Crystals of calcium salts present.

b By aeration.



The jars were opened four months after filling and the condition, appearance, odor, and taste of the silage noted. (See Table I.) With very few exceptions it was in a perfect state of preservation, of excellent texture and color, with a pleasant, somewhat aromatic odor, and generally of an agreeable taste, though quite sour. It was succulent without being too moist, even though it had been made in a tightly sealed jar, with almost no opportunity for the evaporation of water. In order to ascertain its palatability to swine, a representative number of the various mixtures and some of the pure rape silage were fed to three lots of pigs. At first the animals, which were on a ration consisting mainly of corn and tankage, tasted the silage rather hesitatingly and seemed surprised by the sourness, but kept at it until they had eaten it all, appearing to enjoy its succulency. On a second trial, three days later, the same animals ate it with great relish. Only one sample of those tried, a rapemolasses mixture, was refused by the animals. In nearly every case after eating this silage they went to the corn self-feeders. An extensive feeding experiment to determine the effect of feeding rape silage upon the growth and well-being of swine is contemplated.

#### CHEMICAL EXAMINATION OF SILAGE SAMPLES

The data from the chemical examination of the samples are shown in Table I. The juice was pressed out from the silage, and samples were taken from the juice with pipettes. This method has been used with corn silage and is a quite accurate and excellent comparative method for quickly determining the character of a sample of silage. Estimations of the total acidity and moisture content were made on all samples, and estimations of volatile acidity, alcohol, and amino nitrogen on a few representative samples, according to the following methods. (See Table I.)

**TOTAL ACIDITY.**—Ten c. c. of juice were diluted to about 500 c. c. with carbon-dioxid-(CO<sub>2</sub>)-free water, and titrated with decinormal barium-hydroxid solution in the presence of phenolphthalein till a distinct pink appeared by reflected light against a white background.

**VOLATILE ACIDITY.**—Fifty c. c. of juice were diluted to 100 c. c. with carbon-dioxid-free water and distilled with a current of carbon-dioxid-free steam. To hasten the liberation of volatile acids and alcohols, 100 gm. of sodium chlorid were added to the juice. About 500 c. c. of distillate were titrated with baryta water in the presence of phenolphthalein.

**ALCOHOL.**—Distillation method: The distillate from the volatile-acid determination was neutralized with baryta water (solid phenolphthalein being added) and concentrated by repeated distillation with sodium chlorid.<sup>1</sup>

About 50 c. c. of alcohol solution were oxidized<sup>2</sup> in a pressure flask in a boiling water bath for 30 to 40 minutes, and the volatile acids then distilled off four or five

<sup>1</sup> Bacon, R. F. Detection and determination of small quantities of ethyl and methyl alcohol and of formic acid. U. S. Dept. Agr. Bur. Chem. Circ. 74, 8 p. 1911.

<sup>2</sup> The oxidizing solution used was made up in the following proportions: 10 gm. K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>, 20 gm. H<sub>2</sub>SO<sub>4</sub>, 70 gm. water.

times, with additions of carbon-dioxid-free water. The total alcohols found were calculated as ethyl alcohol.

**Aeration method:** In this method a current of air was drawn through the alcohol solution, which was saturated with ammonium sulphate, into concentrated sulphuric acid. The sulphuric-acid solution was then oxidized with potassium-dichromate solution and distilled as before.

**AMINO NITROGEN.**—The amino nitrogen was determined on the diluted juice with the Van Slyke apparatus.<sup>1</sup>

**MOISTURE.**—The moisture content was determined by heating a sample of about 100 gm. in an oven at 100° C.

#### DISCUSSION

The determinations of total acidity, volatile acidity, total alcohols, and amino nitrogen furnish a measure of the most characteristic changes which take place in silage fermentation and a partially complete picture of the character of the fermentation and the character of the silage, as nearly as chemical analysis can show. This, the ordinary estimations of crude protein, fiber, ether extract, and ash fail to do. The amount of amino nitrogen is, of course, of comparative value only, but it shows the degree of hydrolysis of protein. Unfortunately in this case no figures are now available for the amino nitrogen of green rape. The results given in Table I, however, indicate that the degree of hydrolysis of protein was nearly the same in each sample upon which this determination was made. The total acidity was quite similar in each of the samples which were classed "A" and "B." The total acidity of the silage juice in most cases is no higher than the average acidity of corn-silage juice. The average of analyses on 100 c. c. of juice of several samples of normal corn silage is as follows:

Total acidity.....	271 c. c. of N/10 solution.
Volatile acidity.....	91 c. c. of N/10 solution.
Alcohol.....	0.312 gm.
Amino nitrogen.....	0.109 gm.

The explanation of the very sour taste of rape silage may lie in the fact that it has a much higher water content than corn silage and thus affects the nerves of taste more quickly. A considerable amount of sulphates was found in one sample, but the presence of any free mineral acid could not be demonstrated. The volatile acidity seemed to vary more widely, with varying experimental conditions. The alcohol content was probably small in all cases where there was no addition of sugar. In two cases of silage with added sugar or molasses Table I shows that an abnormally large amount of alcohol was found. This, as well as the increased acidity, militates against the addition of molasses to silage materials. It is very probable that the excess alcohol was formed after the maximum acidity had been reached and the yeasts had gained the ascendancy.

<sup>1</sup> Van Slyke, D. D. The quantitative determination of aliphatic amino groups, II. *In Jour. Biol. Chem.*, v. 12, no. 2, p. 275-284, 1 fig., 1 pl., 1 tab. 1912.

The classification of the samples as to general silage quality (A, B, C, etc.) is necessarily approximate. All the samples, however, could be classed as "good silage," except those rated below "D." Those containing fibrous material, such as sorghum cane, Sudan grass, timothy, and corn plant, would be useful for cattle, but would not be as good feed for swine as pure rape silage, or the alfalfa, red clover, potato, or corn-grain mixtures. The silage made from the entire rape plant was quite similar to that made from the leaves. However, for swine too much fiber is objectionable.

The mixtures of rape with legumes are perhaps best, from the standpoint of feeding as well as that of the quality of the silage. The rape improves the mixture, in that it supplies the necessary fermentable carbohydrates, which apparently are deficient in amount in the legume. In this connection it may be noted that since legume silage is not entirely satisfactory, it may be greatly improved by adding 20 per cent or more of rape, which would supply the necessary sugars. On general considerations the indications are that this sort of silage should be useful for either cattle, sheep, or swine. Practical farmers have sowed rape in the corn-field at the time of the last cultivation, it later being ensiled with the corn. This mixed silage has been fed to cattle with apparently good results.

#### SUMMARY

- (1) Rape was successfully ensiled in glass jars, alone and in mixtures with other materials.
- (2) Excepting one or two mixtures, this silage was palatable to swine.
- (3) Chemical examination of the samples showed the acidity and alcohol content to be comparable in most cases to that of corn silage.
- (4) A mixture of rape and a legume produces the best quality of silage.



# EFFECT OF AUTOLYSIS UPON MUSCLE CREATIN

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## INTRODUCTION

The question as to the relation between muscle creatin and urinary creatinin is one which has been the subject of considerable investigation, particularly during the past few years. The importance of this problem lies in the fact that it is now quite clearly established that the creatinin excreted in the urine with a creatin-creatinin-free diet, is an accurate measure of endogenous metabolism. In the mammalian family, creatin is found chiefly in the striated muscular tissue, and to a lesser extent in other tissues and in fluids. The anhydrid creatinin is present in very small quantities. Since the amount of creatinin excreted in the urine is an accurate measure of tissue metabolism and since creatin is a normal constituent of muscular tissue, and since also there is a close chemical relationship between the two compounds, the natural supposition is that urinary creatinin is derived from muscle creatin. This may be said to be the generally accepted view, and it is supported by considerable experimental evidence; yet, on the other hand, certain investigators have obtained results which do not appear to support this theory.

The question as to where creatinin is formed in the body is another problem concerning which there is considerable lack of agreement. In the light of our present knowledge on the subject it must be admitted that the method and the place of production of creatinin in the body have not been clearly established.

In the course of a series of autolytic experiments with lean beef, carried on in connection with investigations concerning changes taking place in beef in cold storage, certain changes were noted in the creatin and creatinin content of the muscles which appear to throw some light on the question as to the source and method of production of creatinin. The results of these observations are offered as a contribution to our knowledge of the subject.

## PREVIOUS AUTOLYTIC EXPERIMENTS

Gottlieb and Stangassinger (3)<sup>2</sup> carried on an extensive series of autolytic experiments with various organs, tissues, and fluids of dogs, cats, and calves, using toluol as an antiseptic. As a result of their studies these

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<sup>1</sup> The authors desire to extend their thanks to Mr. W. C. Powick for assistance rendered in connection with the analytical work reported in this paper.

<sup>2</sup> Reference is made by number to "Literature cited," p. 546.

authors came to the following conclusions: (1) Muscles and other tissues produce creatin in the early stages of autolysis; (2) natural and added creatin are changed in part to creatinin, owing to the action of dehydrating ferments; (3) creatin and creatinin are in part destroyed as autolysis progresses, owing to the action of ferments which they name "kreatase" and "kreatinase."

The work of these authors, so far as changes in free creatinin are concerned, is open to criticism on account of the method which they used for the determination of this constituent. The extracts were concentrated nearly to dryness on a steam bath, the solutions having been neutralized by the addition of barium carbonate. It is now recognized that such a method of concentrating a solution containing creatin will convert a part of that base into creatinin. For these reasons the work of Gottlieb and Stangassinger concerning the production of free creatinin during autolysis must be regarded as of doubtful value.

Stangassinger (9) studied the action of autolyzing body tissues and fluids upon added creatin and the effect of various chemicals and conditions upon the rate and extent of the reaction. Blood, kidneys, livers, and lungs of dogs were used in the experiments. The so-called dehydrating ferments kreatase and kreatinase were found to be most active in weak acid solutions, and toluol had the least retarding action of all the antiseptics used. Protoplasmic poisons checked the action of the ferments. Creatin was formed in the early stages of the autolysis of liver and blood, the material from well-fed animals containing larger amounts of creatin-forming material than that from hungry dogs. Liver extract destroyed added creatinin in appreciable quantities.

This author's findings concerning changes in free creatinin are open to the same criticisms as those made of the work of Gottlieb and Stangassinger (3).

Mellanby (4) carried on autolytic experiments with various tissues, but was unable to confirm in any respect Gottlieb and Stangassinger's findings (3) concerning the effect of autolysis of tissues upon creatin and creatinin. A careful examination of Mellanby's article indicates that his conclusions should not be taken too seriously. For example, rabbit muscle was autolyzed, under strictly aseptic conditions, for five days at 37° C., and at the end of that time no free creatinin could be detected. If it could not then be detected, it certainly could not be found in the author's other experiments, in which autolysis was carried on under less favorable conditions.

Rothmann (7) carried on a series of autolytic experiments in reply to Mellanby's criticism (4) of Gottlieb and Stangassinger's work (3). The work was conducted under strict bacteriological control and it was found that the liver, kidney, and blood of dogs destroyed appreciable quantities of creatin. He admits the correctness of Mellanby's criticism of Gottlieb and Stangassinger's method for the determination of free

creatinin, stating that in the operation creatin was probably changed in part to creatinin. However, using Mellanby's method for the determination of free creatinin, he found that liver and kidney extracts converted appreciable quantities of creatin into creatinin.

Pekelharing and Van Hoogenhuyze (6) found fairly marked increases in the creatin content of muscles on the completion of rigor mortis and heat rigor.

Rowe (8) carried on autolytic experiments with the parathyroids and adrenals of sheep and found that, in a marked degree, these tissues had the property of destroying added creatin. Thyroid extract destroyed 71 per cent of the added creatin in 48 hours and adrenal extract destroyed 69 per cent in 72 hours.

Myers and Fine (5) studied the effect of autolysis upon the creatin and creatinin content of various tissues and fluids of vertebrate animals. Very marked increases were noted in the creatinin content of all the materials examined after autolysis. Human blood and rabbit liver showed marked gains in the total creatinin. In the case of dog muscle an appreciable decrease in total creatinin was noted. The authors are of the opinion that muscular tissue is the site of creatinin formation.

#### THE PRESENT EXPERIMENTS

Two series of autolytic experiments were carried on: One under aseptic conditions; the other with the use of antiseptics. It is generally recognized that the aseptic method is to be preferred, so far as the value of the results is concerned; but owing to the extreme care required in carrying on an autolytic experiment under aseptic conditions, the antiseptic method is commonly employed. In these investigations the antiseptic method was used simply as a check against the aseptic method, and for the purpose of comparison.

#### ASEPTIC AUTOLYSIS EXPERIMENTS

A prime steer was slaughtered at a local abattoir by the usual methods under the personal supervision of one of the authors. It was, of course, impossible to carry out the operation of skinning under strictly aseptic conditions, so the chief aim was to make this operation as cleanly as possible. The entire carcass was first wet down to prevent the dissemination of dust particles. The carcass was kept suspended while it was being skinned and was not allowed to come into contact with the floor, which had also been washed to prevent dust from rising. In skinning the carcass, knives were used which had been dipped in boiling water, and they were again dipped from time to time in the boiling water. As soon as the skin was removed one of the hindquarters was wound with gauze which had been wrung out in a solution of mercuric chlorid (1:1,000); then it was separated from the body and completely enveloped in the

gauze. The hindquarter was next wrapped in dry cheesecloth and heavy paper and transported at once to the laboratory by motor truck, the trip requiring less than an hour.

#### METHOD OF TAKING SAMPLES

To obtain relatively large and aseptic samples of meat such as were used in these experiments is not an easy matter, and extreme care had to be taken to prevent bacterial contamination. After several failures samples free from bacteria were obtained in the following manner: At the laboratory the hindquarter was transferred at once to a special inoculating room about 10 feet square. The walls and floors of this room had been previously washed with the mercuric-chlorid solution. A special canopy ceiling consisting of cheesecloth tacked on a light frame had been placed in the room at the height of about 10 feet, and this was sprayed with a solution of liquor cresolis compositus just before taking the samples. The floor and walls were also sprayed at the same time with the compound cresol solution and were damp while the samples were being taken, the idea being to have the floor, walls, and ceiling moist, so that any floating dust particles would stick to them.

For taking the samples a number of large, heavy-bladed scalpels and long dissecting forceps were used; these had been sterilized and wrapped in cotton. Large plugs of meat, approximating 3-inch to 4-inch cubes, were cut from the muscular tissue, avoiding connective tissue and fat as much as possible. These plugs, weighing from 274 to 512 gm., the average being 377 gm., were immediately transferred to sterile crystallizing dishes fitted with deep glass covers. In cutting out the plugs the line of incision was first thoroughly seared with a hot spatula. Then a light cut was made through the outside to the depth of about 0.5 cm. and the knife used for the incision was laid aside. A second sterile knife was then used for continuing the deeper incision. This was done in order not to carry in any of the mercuric-chlorid solution which might have adhered to the outside. The outer or exposed portions of the meat samples were always trimmed away to the depth of at least half an inch in order to eliminate those portions which had come in contact with the bichlorid gauze. Thirty-three samples were taken in this manner.

The dishes containing the samples were weighed, the covers sealed with adhesive tape, and over the tape were placed strips of tin foil. This was done for the double purpose of preventing evaporation and the possibility of bacterial contamination from the outside.

#### BACTERIOLOGICAL CONTROL OF SAMPLES

The dishes containing the meat samples were placed in the incubator and carefully watched from day to day for evidence of bacterial growth.

Twenty-four of the thirty-three samples showed bacterial contamination upon incubation—that is, visible bacterial growths developed on



the moist surface of the samples, which furnished a good culture medium for bacterial growth. These samples were, of course, rejected. The remaining samples showed no visible bacterial growths upon incubation and were removed from the incubator one at a time after intervals ranging from 7 to 100 days and subjected to a bacteriological examination.

In examining the samples bacteriologically, aerobic and anaerobic cultures were first made from the exuded juice. With sterile instruments bits of muscular tissue were then cut from the outside of the samples and used for cultures. The samples, which, as before stated, consisted of large rectangular pieces approximating 3-inch cubes, were then cut in two with sterile instruments and cultures made by taking bits of the muscular tissue from the center of the samples. A half dozen or more cultures were taken from each sample. Smear preparations were also made from the exuded juice and from the outer and inner portions of the samples and were stained for bacteria.

Upon bacteriological examination nine of the samples were passed as sterile, there being no growths in any of the cultures made from these samples and the smear preparations being negative. These samples were then subjected to chemical analysis (Table I).

The fact that so large a proportion of the samples, 24 out of 33, or about 72 per cent, developed bacterial growths goes to show how difficult it is to obtain sterile samples of meat.

#### METHODS OF CHEMICAL ANALYSIS

After having taken the samples of muscular tissue for incubation the remainder of the quarter of beef was placed in cold storage at 33° F. for 17 hours, when a composite sample of the lean meat was taken for analysis. Analytical work was started about 24 hours after the slaughter of the animal.

All samples of meat, both fresh and after incubation, were finely ground, placed in glass jars, tightly sealed, and analytical work was started promptly.

Moisture and fat determinations were made on all samples. Moisture was determined by drying the material in vacuo over sulphuric acid, and fat was determined in the dry residue by extraction with ether.

PREPARATION OF EXTRACT.—A 0.9 per cent solution of sodium chlorid, saturated with thymol, was used as a solvent. One hundred gm. of the finely ground tissue were macerated in a mortar with the salt solution until a mixture of uniform consistency was obtained. The material was then transferred to a 2-liter volumetric flask, made to volume with the salt solution, and shaken at intervals during a total extraction period of 24 hours. The mixture was then filtered and analytical work begun immediately. Extractions were made in duplicate and the work was carried on in a refrigerated room at a temperature of about 35° F.

ACIDITY was determined by titrating 50 c. c. of the filtered extract against standard sodium-hydroxid solution, using phenolphthalein as an indicator. The results are calculated in terms of lactic acid.

TOTAL CREATININ was determined according to the method of Folin as modified by Emmett and Grindley (1, p. 515). The results are calculated in terms of creatinin.

FREE CREATININ was determined essentially according to the method of Folin (2). Standard creatinin solutions were made from creatinin which had been standardized against  $N/2$  potassium bichromate. With close attention to all details this method was found to give very satisfactory results.

Table I shows the changes in the creatin and creatinin content of lean beef autolyzed under aseptic conditions for periods ranging from 7 to 100 days.

TABLE I.—Changes in creatin and creatinin content of muscle during aseptic autolysis at 37° C.

Serial No.	Incubation period.	Percentage of acid as lactic.	Percentage of total creatinin.	Percentage of free creatinin.	Percentage of creatin calculated as creatinin.	Percentage of total creatinin as free creatinin.
	Days.					
I09.....	.....	3.15	1.73	0.036	1.694	2.08
I10.....	7	3.03	1.97	.422	1.548	21.42
I11.....	14	3.15	1.91	.603	1.307	31.57
I12.....	21	3.16	1.91	.700	1.204	36.90
I13.....	28	4.75	1.63	.756	.774	46.38
I20.....	42	4.33	1.64	.761	.879	46.40
I21.....	64	4.53	1.55	.670	.880	43.23
I22.....	77	5.02	1.62	.742	.878	45.80
I24.....	93	4.74	1.54	.707	.833	45.91
I25.....	100	4.76	1.68	.728	.952	43.33

Changes in total creatinin are fairly marked. Samples incubated for 7, 14, and 21 days show increases in total creatinin amounting to 0.24, 0.18, and 0.18 per cent, respectively. Samples incubated for longer periods, ranging from 28 to 100 days, show appreciable losses in total creatinin varying from 0.19 to 0.05 per cent. On the whole, these data show first an increase in total creatinin and later a decrease, the increases being somewhat larger than the decreases.

The changes in the free creatinin are very marked. The fresh material contains 0.036 per cent of free creatinin, while the sample incubated 7 days contains 0.422 per cent, an actual increase of 0.386 per cent, or a relative increase of 1,722 per cent. Samples incubated for 14, 21, 28, and 42 days show further increases in free creatinin, but the rate of increase is less rapid with each succeeding period. The maximum percentage of free creatinin, amounting to 0.761 per cent, is found in case of the sample incubated 42 days. This is an actual increase of 0.725 per

cent of creatinin as compared with the fresh material. Samples incubated for periods ranging from 64 to 100 days show slight and irregular decreases in free creatinin as compared with the sample incubated 42 days.

The creatin content of the samples, which is calculated by subtracting the percentage of free creatinin from that of total creatinin, shows decreases which correspond to the increases in free creatinin.

The relation between the free creatinin and total creatinin is of special interest. The fresh material contains 2.08 per cent of the total creatinin in the form of free creatinin, while in case of the sample incubated for 7 days the percentage has increased to 21.42. The increases in succeeding periods are less rapid, until a maximum increase is reached in case of the sample incubated 42 days, which contains 46.40 per cent of the total creatinin in the form of free creatinin. However, practically the maximum increase is reached in case of the sample incubated 28 days in which 46.38 per cent of the total creatinin is in the form of free creatinin.

These results show that under the conditions of the experiment an equilibrium is established between the creatinin and creatin. These findings confirm in a remarkable degree results obtained by Myers and Fine (5) in their work with pure solutions of creatin and of creatinin. They incubated solutions of the individual bases for a total period of 337 days, and determined free and total creatinin in each of the solutions at intervals. In case of the solution of creatin, it was found that there was a gradual change of creatin into creatinin until at the end of the period an equilibrium had been established with 44.45 per cent of the total creatinin in the form of free creatinin. In case of the solution of creatinin the change was in the other direction, there being a decrease in creatinin and an increase in creatin, until at the end of 337 days an equilibrium had been established with the relative proportions of creatin and creatinin identical with those noted above.

It is not to be inferred from these findings that the changes which took place in the creatin and creatinin content of muscular tissue during autolysis are entirely natural changes of one base into the other. In case of the autolytic experiments with muscle, practically the maximum change of creatin into creatinin had taken place at the end of 28 days, and nearly half of the total change had taken place in 7 days.

In Myers and Fine's experiments (5) with a solution of pure creatin only 9 per cent of the total creatinin was present in the form of free creatinin at the end of 13 days, and after 53 days only 29 per cent. In our autolytic experiments with muscle, on the other hand, 25.41 per cent of the total creatinin was in the form of free creatinin at the end of 7 days, and 46.31 per cent at the end of 28 days.

It is very evident that the rate of change of creatin into creatinin during the autolysis of beef muscle was greatly accelerated by some agent. The acids in the meat may have facilitated the change in some degree;

but the facts seem to indicate that in considerable part, at least, the change of creatin into creatinin during the autolysis of beef muscle was caused by enzym action.

#### ANTISEPTIC AUTOLYSIS EXPERIMENTS

Muscular tissue, consisting of the pillar of the diaphragm, was obtained from the carcass of a steer immediately after slaughter. The meat was freed from visible fat and connective tissue and finely ground. Thirty-five gm. of meat were weighed into a mortar with 20 gm. of sand, and 50 c. c. of a 0.9 per cent solution of sodium chlorid were added. The tissue was ground to a mass of uniform consistency and then transferred to a 250 c. c. Erlenmyer flask with the aid of 100 c. c. of the salt solution, and the flask was stoppered with a rubber stopper. Sixteen samples were prepared in this manner. After all the samples had been prepared, 2 c. c. each of chloroform and toluol were added to each flask which was then thoroughly shaken. Fourteen of the flasks were then placed in an incubator where they were held at 37° C. for various periods of time. The flasks were shaken daily to insure saturation of the solutions with the antiseptics. Two flasks were placed in a cold-storage room at a temperature of 34° F. and shaken at intervals for a period of 24 hours for the purpose of determining the creatin and creatinin in the fresh material.

#### BACTERIOLOGICAL CONTROL OF SAMPLES

Before adding the antiseptics and before incubation, bacterial counts were made of three of the samples, Nos. 2, 8, and 17, which had been prepared as described above. In making the counts, 0.5 c. c. and 1 c. c. portions of the samples were withdrawn with sterile pipettes and added to tubes of melted agar which were immediately poured into Petri dishes and incubated. The bacterial counts on the three samples were as follows:

Sample 2.....	2,116 bacteria per cubic centimeter.
Sample 8.....	1,486 bacteria per cubic centimeter.
Sample 17.....	1,584 bacteria per cubic centimeter.

The samples were prepared one at a time in the order in which they were numbered—that is, from 1 to 18—and the higher bacterial count in the case of sample 2 is probably due to the fact that this flask was the first one prepared and remained standing for several hours at room temperature, thus giving time for bacterial multiplication before the counts were made. The three counts were made in order to give some idea of the average number of bacteria in the samples before adding the antiseptics and before incubation.

The samples were removed from the incubator for chemical analysis at the intervals given in Table II. In testing the samples bacteriologically, two portions of 1 c. c. each were removed with sterile pipettes and

agar plates made therefrom. In withdrawing the portions for cultures the point of the pipette was introduced well below the surface of the liquid so as to avoid drawing up any of the toluol which floated on the surface. The chloroform, being heavy, settled to the bottom.

In order to avoid carrying over any of the toluol on the pipettes, the ends of the pipettes were washed with sterile, distilled water before their contents were delivered into the agar tubes. A single colony was observed in one of the plates made on the fourth day and single colonies were observed in each of the plates made on the eighth day, but after this the plates remained sterile.

The absence of bacterial development in the plates may have been due to inhibition of growth by small amounts of the antiseptics dissolved in the meat infusion rather than to actual destruction of the organisms present. However, the results seem to afford ample evidence that there was no bacterial multiplication in the samples during the course of the experiment.

#### CHEMICAL STUDIES

Moisture was determined in the fresh material for the purpose of correcting for the volume of water in the meat.

Creatin and creatinin were determined in the filtered extracts from the various samples according to the methods of Folin, as previously noted (2).

Table II shows the changes in the creatin and creatinin content of muscular tissue from the ox incubated at 37° C. in the presence of antiseptics for periods ranging from 2 to 84 days.

TABLE II.—Changes in creatin and creatinin content of beef muscle during antiseptic autolysis at 37° C.

[Expressed as percentages of fresh material.]

Serial No.	Incubation period.	Percentage of total creatinin.	Percentage of free creatinin.	Percentage of creatin calculated as creatinin.	Percentage of total creatinin as free creatinin.
I.....		0.28	0.0047	0.2753	1.67
2.....	48 hours....	.29	.0140	.2760	4.78
4.....	95 hours....	.28	.0195	.2605	7.07
5.....	7 days.....	.29	.033	.257	11.26
6.....	14 days....	.29	.049	.241	16.65
7.....	21 days....	.28	.060	.220	21.09
8.....	28 days....	.27	.067	.203	25.09
9.....	35 days....	.28	.080	.200	28.12
10.....	42 days....	.28	.107	.173	37.61
11.....	49 days....	.28	.098	.182	35.53
12.....	56 days....	.28	.101	.179	36.61
13.....	63 days....	.29	.109	.181	37.19
14.....	70 days....	.29	.112	.178	38.21
15.....	77 days....	.28	.113	.167	39.70
17.....	84 days....	.29	.113	.177	38.53

There are practically no changes in the total creatinin in contrast to the fairly marked changes in this constituent noted in case of the aseptic autolytic experiment. This fact does not indicate that the changes in total creatinin content observed in case of the aseptic autolytic experiment are in error, but rather that the antiseptics used in the second experiment probably prevented the change.

There is a marked increase in free creatinin during the course of the experiment, the increase taking place most rapidly in the early stages of the incubation period, and less rapidly toward the end of the experiment, until finally there was practically no change.

On account of the different bases of calculation, these data can not be compared directly with similar data obtained in case of the aseptic autolytic experiment. However, the general trend of the change in free creatinin is the same in each experiment. In the first experiment a maximum production of free creatinin was reached in 42 days, in the second experiment in 84 days.

Creatin shows decreases corresponding to the increases in creatinin. The data showing changes in the relation of free creatinin to total creatinin indicate most clearly the changes in these constituents during the course of the experiment. The transformation of creatin into creatinin takes place most rapidly during the first 24 hours, and the rate of change steadily decreases during the course of the experiment, until at the end of 77 days a maximum change is reached, the free creatinin then constituting 39.70 per cent of the total creatinin. It is possible that if the experiment had been continued for a much longer time a larger proportion of creatin would have been converted into creatinin. These data confirm the changes of creatin to creatinin observed in the case of the aseptic autolytic experiment, and also the fact that the total extent of the change is limited. In the first experiment a maximum change of 46.40 per cent of total creatin, calculated as creatinin, to creatinin was observed, while in the second experiment the total change amounted to 39.70 per cent.

The results obtained in the antiseptic autolytic experiment confirm those obtained in the experiment conducted under aseptic conditions, both as regards change of creatin into creatinin, and in that the total extent of the reaction is limited, but do not confirm those showing first an increase and later a decrease in total creatinin.

#### DISCUSSION OF RESULTS

The results of the experiments reported in this paper show very clearly the transformation of muscle creatin into creatinin during autolysis. To a very considerable degree this transformation must be regarded as due to the action of enzymes.

These findings are substantiated by the work of Gottlieb and Stangassinger (3), Stangassinger (9), Rothmann (7), Rowe (8), and Myers and

Fine (5). Mellanby (4) obtained contrary results; but, as has already been noted, a careful examination of his paper indicates something wrong with his work, since he was unable to detect creatinin under conditions in which it was undoubtedly present. His findings should not be taken too seriously. The ability of autolyzing muscular tissue, as well as of other body tissues, to transform creatin into creatinin seems to be quite clearly established.

In the aseptic autolytic experiment there was first an increase in total creatinin and later a decrease as compared with the amount present in the fresh material, while in the experiment carried on under antiseptic conditions there was practically no change. As has been previously noted, it does not follow that the results of the first experiment are in error, but it is possible that in the second experiment the presence of antiseptics prevented these changes in creatin.

A brief examination of the work of previous investigators on this point may throw some light on the question. Gottlieb and Stangassinger (3) observed at first an increase and later a decrease in the total creatinin content of muscular and other body tissues and fluids on autolysis. Stangassinger (9) found an increase in the total creatinin content of blood and liver of dogs and later a decrease in the total creatinin content of the liver. Rothmann (7) found that extracts of the liver and kidney of dogs destroyed added creatinin in a marked degree, and that there was a marked increase in the creatin content of the portal blood of a dog. Rowe (8) observed that extracts from the parathyroid and adrenal glands of sheep destroyed added creatin. Pekelharing and van Hoogenhuyze (6) found an increase in the creatin content of the muscles of dogs after rigor mortis and heat rigor. Myers and Fine (5) found an increase in the total creatinin content of autolyzing human blood and rabbit liver, and a decrease in the total creatinin content of dog muscle. On the whole, the work of these investigators confirms our findings concerning changes in the total creatinin content of beef muscle during aseptic autolysis.

In keeping with the results obtained by Pekelharing and van Hoogenhuyze (6) concerning the effects of rigor mortis upon the creatin content of muscular tissue, it seems very probable that the increase in the total creatinin content of the muscle in our aseptic autolytic experiment was due to the changes accompanying rigor mortis. While analytical work was started 24 hours after the slaughter of the animal, at which time rigor was assumed to be complete, yet in a study of the effects of autolysis upon the soluble muscle proteins changes were observed which indicated that it was not complete at that time.

The establishment of an equilibrium between creatin and creatinin in solutions of the individual bases, as observed by Myers and Fine (5), and our finding as to the establishment of a similar relation between creatin and creatinin in autolyzing muscular tissue, is a matter of more

than passing importance. It denotes, first, that creatin is readily converted into creatinin in pure solution, and, second, that in autolyzing muscular tissue the rate of reaction is very greatly accelerated, but that the total extent of the change is the same in either case. The more rapid change of creatin into creatinin in the autolyzing tissue may safely be assumed to be due, in large part, at least, to enzym action. This conforms to our idea as to the catalytic nature of enzymes. The gradually reduced rate of change of creatin to creatinin during autolysis is in conformity with the law of mass action. These observations are of more interest as regards the chemical relationship of the two substances than on account of their physiological relationship, since in the animal body any change of creatin to creatinin is accompanied with the rapid removal of the creatinin, so that, so far as this factor is concerned, the change always takes place at its maximum velocity. The clear establishment of the fact that muscular tissue has the power in a marked degree of converting creatin into creatinin must be regarded as having an important bearing upon the formation of creatinin in the body. Without going into a discussion of other investigations bearing upon this subject, it may be said that there is much evidence in support of the theory that muscle creatin is the source of urinary creatinin, with a creatin-creatinin-free diet, and considerable evidence to the effect that in part, at least, the transformation of creatin into creatinin takes place in the muscular tissue.

#### SUMMARY

The results of the investigations reported in this paper concerning the effects of autolysis upon the creatin and creatinin content of muscular tissue of the ox may be summarized as follows:

- (1) Muscular tissue has in a marked degree the property of converting creatin into creatinin.
- (2) In the course of autolysis an equilibrium is finally established between creatin and creatinin.
- (3) Muscular tissue appears to have in an appreciable degree the ability both to produce and to destroy creatinin.

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## STORAGE-ROTS OF ECONOMIC AROIDS

By L. L. HARTER,

*Pathologist, Cotton and Truck Crop Disease Investigations, Bureau of Plant Industry*

### INTRODUCTION<sup>1</sup>

The economic aroids within the scope of this article include various species and varieties of the genus *Colocasia* obtained from numerous warm regions throughout the world; a species of *Alocasia* received from Dutch Guiana under the varietal name "Eksi-taya" and *Xanthosoma sagittifolium* (L.) Schott, a native tropical American species. These plants are all of greater or less importance for human food in many tropical and subtropical countries, and they are being grown commercially or experimentally in the southern United States.

The Trinidad dasheen, a variety of taro, gives the greatest promise of success in the United States. It differs from many other taros in that it produces a considerable number of cormels, or "tubers,"<sup>2</sup> of edible size, in addition to the large, edible, central corm. There are a number of varieties resembling it more or less closely. China is believed to have been the original home of the Trinidad dasheen, which is referred to *Colocasia esculenta* (L.) Schott.

Another group of taros, resembling the Trinidad dasheen in general leaf and floral characters and in the production of a large number of tubers, is represented by the Yu-to variety, from Mukden, Manchuria. Several of the Japanese taros, or "imos," are similar to this variety. The tubers are often very numerous, but usually quite small. These varieties are at present also referred to *C. esculenta*.

The Egyptian taro, called "Qolqas," is a member of another group of taros probably belonging to *C. antiquorum* (L.) Schott. A variety of this type, obtained by the Department from Cat Island, S. C., in 1906, is representative of this group. This group is distinguished from the

<sup>1</sup> The first four paragraphs of the introduction were prepared by Mr. R. A. Young, of the Office of Foreign Seed and Plant Introduction, Department of Agriculture.

<sup>2</sup> The word "tuber," the commercial term for "cormel" in the case of the dasheen, is used instead of "cormel" in this paper.

preceding by having a spathe that opens broadly, as well as by the general aspects of the plants. *C. indica* (Lour.) Kunth, a native of Java, was also used in these investigations.

The storage of dasheens by piling the tubers and corms in the field and overlaying them with straw and earth fully protects them against freezes and yields itself readily in other respects to a successful handling of the crop. In these piles, however, unless special means of ventilation are provided, many of the tubers and corms rot so badly as to render them useless for food or propagation. From such decayed material a considerable variety of organisms was isolated during the winter of 1912 and 1913. From similar material about the same organisms were isolated the following year. With these organisms inoculation experiments were made during the winter of 1913 and 1914, and repeated again in 1914 and 1915. Out of the different organisms isolated four were found to be wound parasites under certain conditions. Macroscopically it is not always easy to distinguish the different rots, since in some cases more than one of the rot-producing organisms may be present. An accurate diagnosis is also frequently obscured or rendered difficult by the invasion of saprophytic bacteria and fungi. Furthermore, the striking similarity of some of the rots in the earlier stages renders a diagnosis extremely difficult. While the writer can usually distinguish macroscopically typical cases of the several rots in the later stages, the only sure method is the preparation of cultures.

#### JAVA BLACKROT

The most common and destructive of the storage-rots is called the "Java blackrot" because of its resemblance to the Java blackrot of the sweet potato (*Ipomoea batatas*) caused by the same organism, *Diplodia tubericola*. The causal fungus has been isolated repeatedly during a period of three years from a number of varieties. This disease is particularly interesting in view of the fact that different species of the genus *Diplodia* obtained from other hosts widely separated botanically from the dasheen will cause a decay of the latter identical in character.

#### DESCRIPTION OF JAVA BLACKROT

The tissue when first invaded by the fungus is but little or not at all changed in color and is soft, slimy, and stringy. The substance of the corm or tuber becomes pasty and will, if picked up by the forceps, draw out in a threadlike manner. It is often difficult to distinguish the decay caused by the blackrot fungus in the early stages from the decay produced in the initial stages by other organisms without resorting to plate isolations. A little later, however, the tissue becomes slightly pinkish and then gradually turns black, and in this respect differs from the decayed tissue produced by the other organisms. At the same time the rotted portion of the tuber gradually becomes firmer by the escape of moisture.

Plate LXXXI, figures 1 and 2, shows the typical rots of *C. esculenta* and *Alocasia* sp., respectively, produced by the Java blackrot fungus.

The rot progresses slowly. About seven days elapse after inoculation before any noticeable softening of the tuber occurs under optimum conditions and about four to eight weeks are required for complete destruction of the tuber and blackening of the tissue. Finally both the tubers and corms become very dry and hard and are cut by a knife with difficulty. The middle lamella is first dissolved, the hyphæ later penetrating the cell walls and burying themselves among the starch grains. The tissue finally becomes a disorganized mass and powdery when completely dried. Under normal conditions the rot does not produce any, or, at most, only slight shrinking or malformation of the tuber. In fact, a whole tuber may be completely destroyed internally and become black throughout without much external evidence of it. Fruiting bodies later develop, but they are mostly covered by the epidermis and can scarcely be detected without rupturing the surface.

Under natural conditions the corms decay more readily than the tubers, although the latter are frequently met with in storage and succumb easily to artificial inoculation. It is evident from a careful study of material that natural infection originates in the wounds made by breaking the tubers from the corms and at points where the roots are broken off. After becoming established the fungus may spread in all directions without penetrating deeply until the surface of the corm is well covered, and then it may penetrate farther in; or it may cover an area 1 or 2 inches in diameter and push inward to the center in the form of a cylinder.

#### CAUSE OF BLACKROT

The writer has isolated and successfully inoculated into the dasheen species of *Diplodia* from five different hosts, as follows: *D. tubericola* (E. and E.) Taub. from sweet potato; *D. gossypina* Cke. from a dead limb of cotton; *D. macluræ* Speg. from a dead branch of *Toxylon pomiferum* Raf. from New Jersey; *Diplodia* sp. from a limb of *Mangifera indica* from Cuba, furnished by Dr. J. R. Johnston, pathologist of the Cuban Experiment Station, and a species of *Diplodia* from dasheen which, because of its great similarity to *D. tubericola*, is referred to that species. The type of decay produced by these different species is macroscopically the same. It is a well-known fact that there are a great number of different species of *Diplodia* described in the literature, many of which may prove to be identical. No attempt has been made to go into the taxonomy of this group, but it may be of interest to note the points of similarity and difference between the species here studied. The organism isolated from dasheen can not be distinguished in culture from *D. tubericola* from sweet potato. Both develop into stroma in culture and on the host, and the spores differ but little in shape (fig. 1, A, B) and size.

*D. gossypina* has been shown by Edgerton (3) to be primarily a wound parasite of cotton bolls and by Taubenhaus (9)<sup>1</sup> to produce a

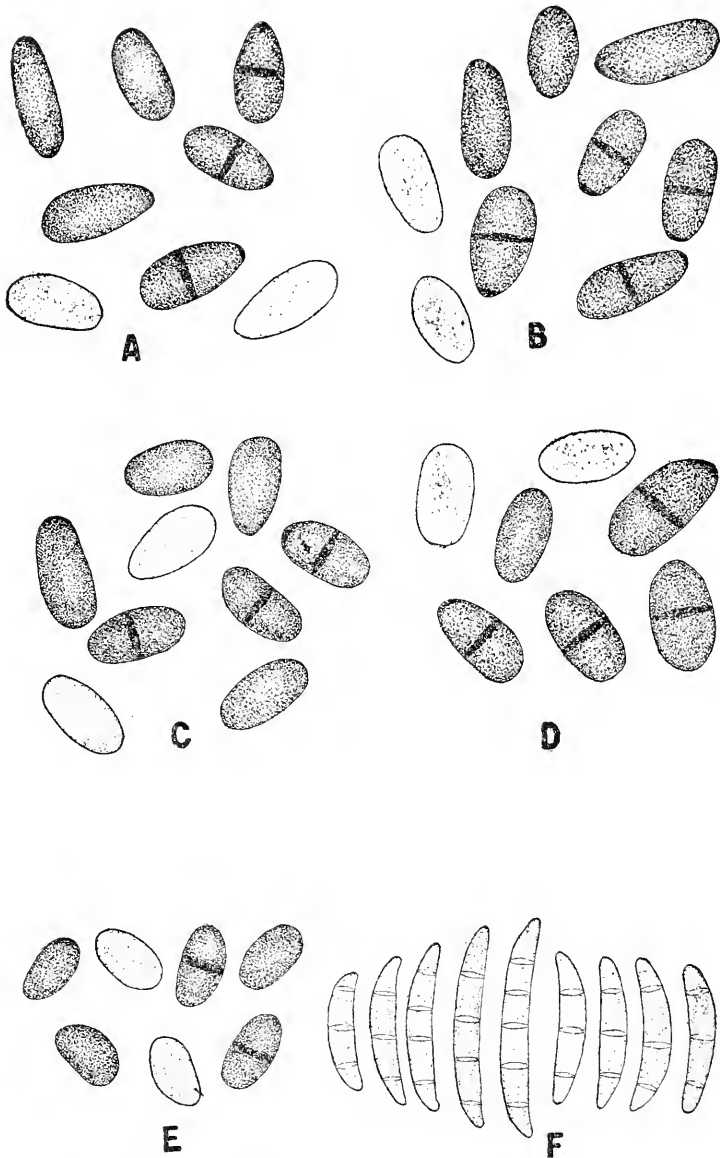


FIG. 1.—Spores of different storage-rot organisms: A, *Diplodia tubericola* from dasheen; B, *Diplodia tubericola* from sweet potato; C, *Diplodia gossypina* from cotton; D, *Diplodia* sp. from *Mangifera indica*; E, *Diplodia macluriae* from *Toxylon pomiferum*; F, *Fusarium solani*.  $\times 500$ .

typical Java blackrot of sweet potatoes. The writer found the fungus produced a decay of dasheens identical with that caused by *D. tuberi-*

<sup>1</sup> Reference is made by number to "Literature cited," p. 571.



*cola* from sweet potatoes and from dasheens and to agree closely in cultural characteristics and in shape (fig. 1, C) and size of spores. While *Diplodia* sp. from *Mangifera indica* and *D. maclurac* both produce a typical rot of dasheens and agree with the other species in cultural characteristics and shape of the spores (fig. 1, D, E), the spores of the latter fungus are uniformly smaller in size. *D. maclurac* is less virulent for dasheens than the other species. The spores from the host of the different species studied measure as follows:

*Diplodia tubericola* from sweet potato, 22.3 to 34.4 by 10.3 to 13.7 $\mu$ . Average, 11.6 by 25.5 $\mu$  (30 measurements).

*Diplodia tubericola* from dasheen, 22 to 33 by 10.3 to 13.7 $\mu$ . Average, 11.3 by 26.5 $\mu$  (30 measurements).

*Diplodia gossypina* from cotton, 20.1 to 28 by 9 to 13.4 $\mu$ . Average, 11.5 by 24.5 $\mu$  (31 measurements).

*Diplodia maclurac* from *Toxylon pomiferum*, 17.5 to 22.3 by 8.5 to 11 $\mu$ . Average, 9.7 by 19.7 $\mu$  (30 measurements).

*Diplodia* sp. from *Mangifera indica*, 23 to 31.6 by 12 to 14.1 $\mu$ . Average, 13 by 26.2 $\mu$  (31 measurements).

In 1906 Charles (2) isolated and studied a species of *Lasiodiplodia* from the fruit of *Mangifera indica*, but left the question unsettled as to whether it was the same organism found on the sweet potato. However, the results obtained by the writer by inoculation studies with the above species and by Taubenhaus (9), who obtained positive infections of sweet potatoes with several species of *Diplodia*, suggest the possible identity of many of these forms described as different species. The results also indicate that these crops are exposed to infection from several sources.

#### INOCULATION EXPERIMENTS

##### INOCULATION OF COLOCASIA ESCULENTA

On January 6, 1914, thirteen dasheen tubers, after being thoroughly washed and disinfected for 10 minutes in mercuric chlorid (1:1,000) and rinsed in water, were inoculated in a wound at the end by inserting spores and hyphæ of *D. tubericola* from dasheen. All inoculations were made from cultures grown on cooked potato cylinders in which spores were present, although in many cases they were hyalin and nonseptate. After inoculation the tubers were placed in a large, uncovered, moist chamber and subjected to the temperature and humidity of the laboratory room. By January 19 the rot had noticeably started on all the tubers, and by January 28 all were completely decayed. The causal organism was recovered in pure culture from each tuber. The checks, six in number, similarly located remained healthy.

On the same date seven tubers prepared as above and inoculated with the same organism were placed in a covered moist chamber with wet filter paper in the bottom and placed on a shelf in the laboratory. These

tubers were kept under observation until February 17, and none showed any evidence of decay. The checks, two in number, under similar conditions but not inoculated, remained healthy. This and subsequent experiments showed that better results could be obtained by merely exposing the inoculated tubers to the surroundings of the laboratory room. The use of moist chambers, therefore, was abandoned, with the exception of an occasional trial experiment to be noted later. Disinfection likewise was no longer practiced, since the tubers were immediately exposed to reinfection from the air of the room. Although the rot caused by *D. tubercicola* is very easily recognized and characteristic when once known, cultures were made from nearly all the decayed tubers, in order to be sure the rot was caused by the used organism. The influence of temperature and moisture on these storage rots will be discussed later.

On January 16, 1914, four tubers were inoculated in the usual way with *D. tubercicola* from dasheen. By February 18 all were rotted and the causal organism recovered in pure culture. The checks, two in number, remained healthy.

On March 1, 1914, twelve tubers were inoculated with *D. tubercicola* from dasheen. On March 12 several tubers showed evidence of decay and by March 20 nine were partially rotted. A portion of some of the tubers was black, and pycnidia containing hyalin 1-celled spores were present. On June 1 all the tubers were completely decayed. The checks, five in number, remained sound.

On January 14, 1915, four tubers were inoculated with *D. tubercicola* from sweet potato. On February 18 all the tubers were rotted, and the causal organism was recovered in pure culture. Two days later ten tubers were inoculated and divided into two equal lots, one being placed in an incubator, the temperature of which varied from 34° to 35° C., and the other in an ice box, the temperature of which varied from 12° to 13°. By February 3 all the tubers in the incubator were rotted and the causal organism was recovered in pure culture, while those in the ice box and the five checks remained sound.

On March 26 six tubers were inoculated with *D. macluræ*. Some time later one was completely decayed and yielded *D. macluræ* in culture; the others remained sound. Four more tubers were inoculated on May 13, 1915, and on June 6 three tubers were half-decayed, *D. macluræ* being recovered from two, *Rhizopus nigricans* from one, and *Fusarium* sp. from one. The checks, five in number, remained sound.

Six other tubers were inoculated on May 20, 1915. On June 1 two were completely decayed and four remained sound.

On December 23, 1914, nine tubers were inoculated with *D. gossypina*, five of which were placed in an open receptacle on the laboratory shelf and four in a moist chamber. All the exposed tubers were rotted on

January 13, and *D. gossypina* was recovered. In the moist chamber two tubers were sound; the other two rotted a very little, one of which yielded *Fusarium solani* and the other *F. oxysporum*. Out of six other tubers inoculated on March 1 five were completely rotted on March 26. The checks, five in all, remained sound.

On January 29, 1915, ten tubers were inoculated with *Diplodia* sp. from *Mangifera indica*. Nine of these tubers showed evidence of rot on February 8; and on February 20 six were completely decayed, three were half-decayed, and one remained sound. The checks, five in number, remained sound.

#### INOCULATIONS OF XANTHOSOMA SAGITTIFOLIUM

On November 30, 1914, five tubers were inoculated with *Diplodia tubericola* from dasheen and five with the same organism from sweet potato. On December 23 all the tubers in both lots were rotted and the causal organism was recovered in pure culture. The checks, five in number, remained sound. Six other tubers were inoculated on December 9 with the sweet-potato organism, and on January 2, 1915, *D. tubericola* was recovered from four and *Fusarium oxysporum* from two.

On January 4, 1915, ten tubers were inoculated with *D. macluræ* and five with *D. gossypina*. By February 10 five of the former and three of the latter were decayed and the causal organisms recovered. The five checks remained sound.

#### INOCULATION OF COLOCASIA INDICA

On December 9, 1914, four tubers were inoculated with *D. tubericola* from dasheen and five with the same organism from sweet potato. All the tubers in both lots were completely decayed on January 2, 1915, and the causal organism was recovered. The checks, four in number, remained sound.

#### INOCULATION OF ALOCASIA SP.

Five tubers were inoculated on January 2 with *D. tubericola* from dasheen, and on February 10 three were decayed. *D. tubericola* was recovered from two and *Fusarium* sp. from one. The two others and the five checks remained sound. On the same day five tubers were inoculated with the same organism from sweet potato, and on February 10 one tuber was sound. The four others were only partially decayed, but *D. tubericola* was recovered from the rotted portion. It appears that, while this species is not wholly immune to the rot, it is more resistant than the others. On January 4, 1915, four tubers were inoculated with *D. macluræ* and four with *D. gossypina*. All those inoculated with *D. macluræ* remained sound, but of those inoculated with *D. gossypina* two were completely decayed and two one-third rotted. The causal organism was recovered from each.

Table I gives the results of these inoculation experiments with *Diplodia* spp.

TABLE I.—Results of the inoculations of tubers of *Colocasia esculenta*, *Xanthosoma sagittifolium*, *C. indica*, and *Alocasia* sp. with *Diplodia tubericola*, *D. macluræ*, *D. gossypina*, and *Diplodia* sp. from *Mangifera indica*

Organism.	<i>Colocasia esculenta</i> .			<i>Xanthosoma sagittifolium</i> .			<i>Colocasia indica</i> .			<i>Alocasia</i> sp.		
	Inoculated.	Infected.	Checks.	Inoculated.	Infected.	Checks.	Inoculated.	Infected.	Checks.	Inoculated.	Infected.	Checks.
<i>D. tubericola</i> from dasheen...	36	29	<sup>a</sup> 13	5	5	<sup>a</sup> 5	4	4	<sup>a</sup> 4	5	5	<sup>a</sup> 5
<i>D. tubericola</i> from sweet potato.....	14	9	5	11	9	5	5	5	4	5	4	.....
<i>D. macluræ</i> .....	10	4	5	10	5	5	.....	.....	.....	4	0	.....
<i>D. gossypina</i> .....	15	10	5	5	3	5	.....	.....	.....	4	4	.....
<i>Diplodia</i> sp. from <i>Mangifera indica</i> .....	10	9	5	.....	.....	.....	.....	.....	.....	.....	.....	.....

<sup>a</sup> None of the checks became infected.

#### POWDERY GRAYROT

Since this form of storage-rot has never been reported before, the writer proposes that it be known by the name "powdery grayrot." This, like many other common names of plant diseases, is somewhat misleading, since the rot in its early stages is soft and, if invaded by bacteria, is slimy on the surface. In the later stages, however, it becomes powdery and gray, this appearance serving to distinguish it from the other storage-rots.

#### DESCRIPTION OF POWDERY GRAYROT

This rot has been isolated repeatedly from tubers and corms from Brooksville, Fla., and from specimens imported from Japan in May, 1915. Infection usually begins in the wounds made by breaking the tubers and corms apart, showing that it is probably strictly a wound parasite. When infected at such a point, the rot may spread rather widely over the surface, penetrating only half an inch or so; or it may penetrate under a small area to the center of the tuber or corm, though the number of specimens having been seen completely decayed by this organism is relatively small. In the final stages this rot becomes rather hard, dry, and powdery and is of a grayish color and crumbles when cut with a knife.

Numerous inoculation experiments have made it possible to study the progress of this rot more in detail in the laboratory. The first evidence of decay appears in 24 hours after inoculation on a cut surface, manifested by the formation of an ochreous to salmon-orange color. This color

becomes gradually darker and eventually turns brown, particularly just below the surface. Softening accompanied by stringiness of the tissue begins in 48 hours and extends to a depth of  $\frac{1}{4}$  to  $\frac{1}{2}$  inch in one week. After a week or 10 days the surface becomes somewhat slimy and glistening from the production of pionnotes composed of numerous typical spores of the causal fungus. Upon drying, the specimen takes on a putty-like texture, shrinks perceptibly, and finally becomes dry and powdery and of a dark-grayish color. Plate LXXXII, figures 1 and 2, shows typical specimens of *Colocasia esculenta* and *Xanthosoma sagittifolium*, respectively, partially decayed by the powdery-grayrot fungus.

An examination of rotted material shows that the fungus first destroys the middle lamella and later to some extent invades the cells themselves, the tissue finally becoming a disorganized mass of separated cells.

#### CAUSE OF POWDERY GRAYROT

For a period of three years *Fusarium solani* (Mart.) Sacc. has been repeatedly isolated in pure culture from decayed tubers and corms and has reproduced the characteristic rot when inoculated into dasheens. From such inoculated tubers the organism has been recovered and again made to produce the disease and subsequently recovered. The causal organism has been found to agree with *F. solani* as laid down by Appel and Wollenweber (1) both culturally and in size and septation (fig. 1, *F*) of spores, as shown by the following measurements: Tri-septate conidia taken from pionnotes of a 16-day-old culture on cooked Irish potato vary from 27 to 41 by 5.0 to 6.2 $\mu$  and average 5.7 by 37.0 $\mu$ . Four-septate conidia, 34.4 to 51.6 by 5.2 to 6.2 $\mu$ , average 5.7 by 41.6 $\mu$ . Five-septate conidia, 5.4 to 5.9 by 41.3 to 51.6 $\mu$ , average 5.6 by 47.4 $\mu$ . In this connection it should be stated also that *F. solani* from Irish potato, isolated and identified by Wollenweber at Dahlem, near Berlin, Germany, produced a similar rot of dasheens. No difference between the two organisms could be detected either culturally or in their parasitic habits.

#### INOCULATION EXPERIMENTS

A few preliminary experiments demonstrated that no decay would result when this fungus was spread on an unbroken surface. On the other hand, if placed on a freshly wounded surface, decay started in 24 to 48 hours, provided sufficient moisture was present to enable the fungus to get a start. These results seem to indicate that the fungus gains access to the tubers through wounds made by separating the tubers and corms or through wounds made by other means. The results of our experiments showed that two reliable methods of inoculation could be trusted; (1) Inoculation of the tuber by wounds made by pricking with a sterile needle or scalpel or (2) by splitting a corm or tuber in two and smearing spores on the cut surface. If the latter method was employed, a

film of water, such as may be supplied by a fine spray from an atomizer, must be provided for one or two days, after which the rot will continue independently. That *F. solani* smeared on a moist cut surface of dasheen develops as a wound parasite and not a saprophyte is evident from the fact that other fungi, such as *F. oxysporum* Schlecht., and *F. caudatum* Wollenw. isolated from dasheen, when similarly used produced no decay.

#### INOCULATION OF COLOCASIA ESCULENTA FROM TRINIDAD

On January 21, 1915, six tubers were inoculated in a moist chamber by smearing spores of *F. solani* from dasheen on the cut surface. A soft-rot started in two days and by January 27 it had penetrated half an inch. The causal fungus was recovered from each. The checks, six in number, remained sound. On January 24, six tubers were inoculated and by February 11 all were completely decayed and *F. solani* was recovered from five. The plate in which the other planting was made was overrun with a species of *Rhizopus*. The checks, six in number, were sound. Six tubers inoculated on February 6 were completely rotted in 9 days. Six inoculations made on March 1, 1915, in the usual way were all rotted on March 9. No isolations were made. The checks, four in all, remained sound. On March 6 four tubers were inoculated into a cut surface at the end of the tuber and two on an unbroken surface at the side. Those inoculated into a wound rotted freely; the others remained sound.

On February 16 six tubers were inoculated with *F. solani* from Irish potato, and in nine days the tubers were well decayed, the rot being identical with that produced by tubers inoculated with the same organism from dasheen. The checks, six in all, remained sound. On March 1 six tubers were inoculated with *F. solani* from Irish potato, and in nine days all the tubers were mostly but not completely rotted. The four checks remained sound.

#### INOCULATIONS OF COLOCASIA ESCULENTA FROM MANCHURIA

This is a variety of taro from Manchuria with small tubers about 2 inches long and 1 inch in diameter. On March 1 twelve of these tubers were inoculated with *F. solani* from dasheen. In seven days all the tubers were completely decayed, and the causal organism recovered from each. The checks, six in number, remained sound. An examination of the decayed specimens showed that, while the middle lamella was largely destroyed, the fungus did not to any extent invade the cells.

#### INOCULATIONS OF XANTHOSOMA SAGITTIFOLIUM

On March 1 six tubers were inoculated with *F. solani* from dasheen, and in seven days the tubers were well decayed but not completely, the rot being identical with the rot of *Colocasia esculenta* produced by the same organism. *F. solani* was recovered from each tuber in pure culture. The checks, four in number, remained sound.

The results of these inoculation experiments with *F. solani* are given in Table II.

TABLE II.—Result of the inoculations of *Colocasia esculenta* (Trinidad), *C. esculenta* (Manchuria), and *Xanthosoma sagittifolium* with *Fusarium solani*

<i>Colocasia esculenta</i> (Trinidad).			<i>Colocasia esculenta</i> (Manchuria).			<i>Xanthosoma sagittifolium</i> .		
Inoculated.	Infected.	Checks.	Inoculated.	Infected.	Checks.	Inoculated.	Infected.	Checks.
a <sub>42</sub>	39	b <sub>26</sub>	12	12	b <sub>6</sub>	6	6	b <sub>4</sub>

a Twelve tubers were inoculated with *F. solani* from Irish potato; all others with *F. solani* from dasheen.  
 b None of the checks became infected.

### SCLEROTIUM-ROT

The sclerotium-rot, while common in the storage heaps where high temperatures and a relatively high humidity prevails, is not so frequently met with under all circumstances as rots caused by *F. solani* and *D. tubercicola*. The causal fungus is known to occur on a number of hosts widely separated in relationship, such as tomato (*Lycopersicon esculentum*), peanut (*Arachis hypogaea*), cabbage (*Brassica oleracea*), cotton (*Gossypium* spp.), violet (*Viola* spp.), and others. It has been found growing on the dead scales and other débris of many dasheen plants in the field in Florida, but not a single sure case has been found where it invaded the sound tissue. It, like the other fungi so far discussed, is primarily important only as a storage-rot.

#### DESCRIPTION OF SCLEROTIUM-ROT

During a period of three years many tubers and corms have been examined which were somewhat mushy and watery and often covered by numerous almost spherical sclerotial bodies. The watery putrid condition often accompanying this decay is usually the result of saprophytic fungi and bacteria which followed the progress of the Sclerotium fungus. If this putrid substance is pared away, a firmer (Pl. LXXXI, fig. 3), almost odorless decay will be found from which a pure culture of the causal organism can be plated out. The rotted tissue is ocherous to brown in color, soft but not watery, with a tendency to stringiness. A sharp line characterized by a difference in color separates the healthy from the diseased tissue. The destruction of the tissue is apparently brought about by an enzym secreted by the fungus. At least there is a soft zone  $\frac{1}{4}$  to  $\frac{1}{2}$  inch in width with the characteristic color of the rot from which the organism can not be isolated.

The hyphae do not enter the cells to any extent, but the tissue finally becomes badly disorganized through the destruction of the middle lamella.

## CAUSE OF SCLEROTIUM-ROT

The sclerotium-rot is caused by *Sclerotium rolfsii* Sacc., a fungus which was first mentioned by Rolfs (6, p. 31) in 1893 and technically described by Saccardo (7, p. 257) in 1911.

In about seven days after inoculation in a moist chamber the sclerotial bodies begin forming. They are almost spherical, at first white, but later becoming brown, and finally nearly black, with a hard, shiny surface. This organism, the sclerotial bodies of which are composed of solid masses of fungus tissue, is, according to Wolf (10), parasitic on peanuts and a number of other legumes.

## INOCULATION EXPERIMENTS

All inoculations were made in moist chambers and kept in the laboratory except those in which temperature relations were studied, the results of which are discussed later. All attempts to produce the rot by placing bits of hyphæ on an unbroken surface of the tuber were unsuccessful. It was later found, however, that when the inoculations were made on a cut surface or in a small wound made by a scalpel they were uniformly successful if sufficient moisture was provided at the outset. Moisture was consequently furnished by spraying once or twice with water from an atomizer, and after 24 to 48 hours further applications of water were unnecessary. The fungus grows very rapidly and in a few days covers the whole surface of a tuber (Pl. LXXXII, fig. 3) split in two and even spreads onto the unwounded surface, although the scales of these aroids appear to be impenetrable by the fungus. Within a week the tissue is softened for half an inch or more, although under favorable conditions a month is often required to decay completely a tuber.

## INOCULATION OF COLOCASIA ESCULENTA

On January 14, 1915, six tubers of the Trinidad dasheen were inoculated with *S. rolfsii* by placing bits of hyphæ on a cut surface. Decay started in 2 days, and in 13 days the hyphæ had overrun the whole cut surface of the tuber and softened the tissue to the depth of half an inch. The checks, six in number, remained sound. On January 27 six tubers were inoculated, and by February 9 the tubers were well rotted and sclerotia forming. The checks, four in number, remained sound. On February 2 sixteen tubers were inoculated, and in 13 days all were softrotted and covered with a dense growth of hyphæ. The checks, five in all, remained sound. On February 6 four tubers were inoculated on an unbroken surface, but no growth had taken place by February 23, and they were thrown out. No checks. On February 15 eight tubers were inoculated and in 8 days they were all soft rotted, with sclerotia developing abundantly. Two tubers were inoculated on March 6 at the end in a small wound made by a scalpel and were well rotted by March 15.



INOCULATION OF XANTHOSOMA SAGITTIFOLIUM

On January 25 sixteen inoculations were made by placing bits of hyphæ on the cut surface and in 12 days all were softrotted and sclerotia abundantly produced. The six checks remained sound.

The results of the inoculation experiments with *S. rolfsii* are given in Table III.

TABLE III.—Results of inoculation experiments with *Sclerotium rolfsii*

Host.	Date of inoculation.	Inoculated.	Infected.	Checks.	Checks infected.
<i>Colocasia esculenta</i> .....	Jan. 14	6	6	6	o
Do.....	Jan. 27	6	6	4	o
Do.....	Feb. 2	16	16	5	o
Do.....	Feb. 6	4	0	0	o
Do.....	Feb. 15	8	8	0	o
Do.....	Mar. 6	2	2	0	o
<i>Xanthosoma sagittifolium</i> .....	Jan. 25	16	16	6	o

SOFTROT

Many tubers have been examined which were softrotted and emitted a very disagreeable, repellent odor. At first the odor was supposed to be produced by saprophytic bacteria following the invasion of the host by some one of the organisms already discussed. From many such specimens, however, after paring away most of the rotted material, no fungi could be isolated. Microscopic examination of such material disclosed very actively motile bacteria which were readily isolated by the poured-plate method.

This is the only disease of the four studied which occurs to some extent in the field, mostly in the lower and poorly drained parts. Plate LXXXIII shows a corm and leaf attached as it appeared when lifted in the field. The lower part of the corm is decayed away. The organism isolated from this corm was used in some of the inoculation experiments which follow. The organism was also isolated from tubers and corms in the storage piles and once from the dark strands running through the corms. These strands sometimes appeared darker than normal, and microscopic examinations indicated invasion by some organism, but repeated attempts to isolate one failed until the winter of 1915, when a bacterium was isolated from a diseased strand in the center of a big corm by macerating bits of the decayed tissue in a tube of sterile water and pouring agar plates in the customary way. Numerous colonies later developed which proved to be identical with that produced by the other strains isolated from rotted tissue and to produce a rot similar to it. Usually these strands can be traced to the exterior of the corm, showing that the invading organism probably followed the strand. Under suit-

able conditions decay sets in which eventually results in the partial or complete destruction of the corm.

#### DESCRIPTION OF SOFTROT

Softrot is characterized by being watery and slimy, with a disagreeable, repellent odor. The tissue is little or not at all changed in color under natural conditions. Under sterile, artificial conditions the surface becomes slightly reddish brown. Sections through diseased tissue show that the middle lamella is dissolved and the intercellular spaces are filled with bacteria. The cells themselves are seldom, if ever, invaded.

#### CAUSE OF SOFTROT

The softrot of dasheen is caused by the well-known softrot organism of many vegetables, *Bacillus carotovorus* Jones. This conclusion was arrived at by a comparison in culture of the growth of the organism from dasheen with an authentic culture of *B. carotovorus* kindly furnished by Dr. L. R. Jones, of the University of Wisconsin, and by a series of cross-inoculations.

The comparison of growth of *B. carotovorus* on different culture media was made with three strains from dasheen as follows:

3624. *Bacillus carotovorus* Jones (furnished by Dr. Jones).

3595. A strain isolated from a partially softrotted Trinidad dasheen.

3616. A strain isolated from a Pat-long-fu taro (*C. esculenta*). (See Plate LXXXIII.)

3626. A strain isolated from the fibrovascular bundles at the center of a big corm of a Trinidad dasheen.

All these strains have produced the typical decay by inoculation. After rejuvenating the strains by transferring for several consecutive days to beef bouillon the following culture media were inoculated: Potato cylinders, milk, litmus milk, gelatin, nitrate solution, Cohn's solution, Dunham solution, Uschinsky's solution, beef bouillon, beef-agar slants, beef-agar plates and saccharose, lactose, dextrose, and glycerin bouillon in fermentation tubes. None of the strains grew in Cohn's solution. Gelatin was promptly liquefied by all strains, and nitrates were changed to nitrites when tested according to the method recommended by Smith (8).

Strain 3624 gave a prompt test for indol upon the addition of sulphuric acid and sodium nitrate, white strain 3595 yielded but a faint pink at first, which intensified upon warming to 75° C. The other two strains were doubtful. Strain 3624 was a slower grower than the others on practically all media as well as the less vigorous parasite, but the difference between the growths of this strain on the various media was no greater than the difference between the growths of the different strains from dasheens, or between the growths in different tubes of the

same strain. The one striking exception to the above statement may be noted in connection with the results obtained with saccharose, lactose, dextrose, and glycerin broth in fermentation tubes. Strain 3624 produced gas (a small amount) in all, while none of the other strains did. Such a difference, however, is not surprising in view of the fact that Harding and Morse (4) found that of the various strains from different sources studied by them some consistently failed to produce gas.

The writer wishes to emphasize in this connection that he has carefully compared his results with the studies of Jones (5) and Harding and Morse (4) and has frequently consulted Smith's "Bacteria in Relation to Plant Diseases" (8) for methods. Slight differences in cultural characteristics have been noted from time to time between the different strains, but these differences appear to be no greater than would naturally be expected between strains of the same organism. No attempt has been made to duplicate all the work of Jones or of Harding and Morse with this group of organisms, but merely to carry the work of comparison far enough to be reasonably sure that the writer was working with a strain similar to or identical with *B. carotovorus*.

By a series of cross-inoculations it was shown that the organism furnished by Dr. Jones would decay dasheens and the organisms from dasheens softrotted raw carrots and turnips. It should be emphasized in this connection that strain 3624 (Jones) was less virulent for dasheens than the strain isolated from dasheen, though it rotted carrots and turnips with ease.

#### INOCULATION EXPERIMENTS

As a preliminary test, 12 sterile raw blocks in test tubes with a little water added were cut from corms and inoculated on April 1, 1915, with a 24-hour-old culture (organism 3595) on beef bouillon. In three days there was evidence of decay in some of the tubes, and in 10 days four of the blocks were completely rotted. The checks, six in number, remained sound. The causal organism was recovered in pure culture from two of the blocks.

On April 19, 1915, twelve more sterile raw blocks and also six dasheen tubers in moist chambers were inoculated with a 3-day-old culture of beef bouillon by placing a loopful of the broth in a depression of a cut surface. The raw blocks in test tubes were all decayed by April 24. Four of the tubers in moist chambers were well rotted on the same date and the other two but slightly. The causal organism was reisolated from four. Six raw blocks in tubes and three tubers in moist chambers were held as checks. All remained sound. The lack of material prevented further work at this time. The work was again taken up in November, the tubers or corms being cut in two and inoculation made on the wounded surface in moist chambers, the surface being kept moist for

a day or two by spraying with sterile water from an atomizer. Cultures from beef bouillon were used for all inoculations.

On November 27 eight tubers were inoculated with organism 3616 from a 3-day-old culture and all were completely decayed in seven days. The organism was recovered from four. Strain 3616 was isolated from a Pat-long-fu taro (*Colocasia esculenta*) on November 18, 1915. The corm was decayed at the base and was lifted a few days before in the condition shown by Plate LXXXIII. Ten raw sterile blocks inoculated on the same day with each of strains 3595 and 3616 were completely decayed in three days. On November 24 ten tubers were inoculated with organism 3624 (*Bacillus carotovorus* from Dr. L. R. Jones) from a 24-hour-old culture. A slight rot had taken place in seven days; and in 12 days, although the decay had increased, it was still slight. The causal organism was recovered from three tubers. On December 1 eight tubers were inoculated with strain 3624, and in five days about half of each tuber was decayed. The organism was recovered from four. The checks, six in number, remained sound. On December 9 six raw carrot and six raw turnip blocks in test tubes were inoculated with strains 3616 and 3595. Decay started in 24 hours and was complete in five days. At the same time thirteen raw blocks of dasheens were inoculated with strain 3624 from a 2-day-old culture, and in 5 days nine blocks were completely decayed; the others and the ten checks remained sound. On December 13 three turnips and three carrots in moist chambers were inoculated with a 6-day-old culture of strain 3595, and by December 20 two turnips and one carrot were completely decayed. At the same time six dasheen tubers were inoculated with a 6-day-old culture of strain 3624. Decay began in 24 hours and in seven days had destroyed most of each tuber. The four checks remained sound. On December 20, 1915, four turnips and four carrots were inoculated with a 7-day-old culture of organism 3627 (a re-isolation of 3616) and three turnips and four carrots with strain 3595. A rot started in 48 hours and decay was complete in seven days. The causal organism was recovered from the four turnips inoculated with strain 3627 and from the four carrots inoculated with strain 3595. The ten checks remained sound.

On December 27 four dasheens were inoculated with 1-day-old cultures of strain 3616 and six carrots and seven turnips with strain 3624. By January 3 the dasheens were nearly decayed and all the turnips and five of the carrots completely rotted. All the checks remained sound. Eleven turnips inoculated with 1-day-old cultures of strain 3616 were completely decayed by January 3, 1916. Four dasheen corms inoculated with 1-day-old culture of 3624 were but slightly rotted at the end of seven days. On December 28 three turnips and four carrots were inoculated with a 24-hour-old culture of organism 3626 (an isolation from a fibrovascular bundle at the center of a large corm). In six days

all but one turnip was nearly rotted. The four checks remained sound. On December 31 five turnips and five carrots were inoculated with a 48-hour-old culture of organism 3627. By January 9, 1916, three turnips and four carrots were badly rotted. The organism was recovered from all the decayed specimens. On January 6, 1916, four dasheen corms were inoculated with a 3-day-old culture of strain 3624. By January 13 a slight rot had taken place. The rot progressed but little in one more week and the specimens were thrown out. On January 7 six turnips and six carrots were inoculated with a 1-day-old culture of strain 3627 and by January 12 all were completely decayed. The causal organism was received from six. The seven checks remained sound.

Table IV gives the results of the inoculation experiments with *Bacillus carotovorus*.

TABLE IV.—Results of inoculation experiments with *Bacillus carotovorus*

Strain No.	Date of inoculation.	Host.			Inoculated.	Infected.	Checks.	Checks infected.	Reisolations.
		Dasheen.	Turnip.	Carrot.					
	1915.								
3595	Apr. 1	Raw blocks			12	4	6	0	2
3595	Apr. 19	do			12	12	6	0	0
3595	do	Tubers			6	4	3	0	0
3595	Nov. 27	Raw blocks			10	10	0	0	4
3595	Dec. 9		Raw blocks		6	6	10	0	0
3595	Dec. 13		Roots		3	2	0	0	0
3595	Dec. 20		do		3	3	10	0	0
3595	Dec. 9			Raw blocks	6	6	10	0	0
3595	Dec. 13			Roots	3	1	0	0	0
3595	Dec. 20			do	4	4	10	0	4
3616	Nov. 27	Tubers			8	8	0	0	4
3616	do	Raw blocks			10	10	0	0	0
3616	Dec. 27	Tubers			4	4	2	0	0
3616	Dec. 9		Raw blocks		6	6	10	0	0
3616	Dec. 27		Roots		11	11	10	0	0
3616	Dec. 9			Raw blocks	6	6	10	0	0
3624	Nov. 27	Tubers			10	<sup>a</sup> 10	0	0	3
3624	Dec. 1	do			8	8	6	0	4
3624	Dec. 9	Raw blocks			13	9	10	0	0
3624	Dec. 13	Tubers			6	6	4	0	0
3624	Dec. 27		Roots		6	6	10	0	0
3624	do			Roots	7	5	10	0	0
3626	Dec. 28		Roots		3	2	4	0	0
3626	do			Roots	4	4	4	0	0
3627	Dec. 20		Roots		4	4	10	0	4
3627	Dec. 31		do		5	3	0	0	3
3627	Dec. 20			Roots	4	4	10	0	4
3627	Dec. 31			do	5	4	0	0	4
	1916.								
3624	Jan. 6	Tubers			4	<sup>a</sup> 4	0	0	0
3627	Jan. 7			Roots	6	6	3	0	3
3627	do			Roots	6	6	4	0	3

<sup>a</sup> All slightly.

## OTHER FUNGI ISOLATED AND STUDIED

In storing on the ground a crop such as dasheens it is only natural that a number of saprophytes would be associated with the storage-rot organism. Two species of *Fusarium*, *F. oxysporum* and *F. caudatum*, were frequently isolated under such conditions. Although preliminary inoculation experiments made by inserting spores and hyphæ of these organisms with a needle or by smearing spores on a cut surface of the tubers in a moist chamber gave negative results, it was still believed that they would produce true storage-rots under the proper conditions. As the writer believed that sufficient moisture was lacking, the tubers, after being dipped in spore suspension in sterile water, were wrapped with wet filter paper and then with oiled paper and placed in a moist chamber. The results were negative. Tubers soaked for one hour in water and then dipped in a spore suspension and wrapped in filter paper and oiled paper remained sound. Again, tubers kept at a temperature of about 12° C. for 10 days, inoculated and manipulated as above, and kept in a moist chamber yielded no result. It was finally concluded from these results, in view of the fact that other organisms readily cause storage-rots under laboratory conditions, that these two fungi were merely saprophytes. A species of *Phomopsis* isolated from dasheens from the Hawaiian Islands failed to produce a rot under any of the conditions tried. Other fungi isolated a few times but not studied were *Rhizopus nigricans*, *Penicillium* spp., *Pythium debaryanum*, *Fusarium redolens*, and an undetermined species of *Fusarium*.

A number of inoculations were made with *Diplodia zeeae*, *Sphaeropsis malorum*, and a species of *Diplodia* from salix, none of which produced a rot.

## MOISTURE AS A FACTOR IN PRODUCING ROT

It is likely that moisture plays a far greater part in the production of storage-rots than is generally conceded. Ordinarily it might be supposed that the amount of humidity in a moist chamber lined with saturated filter paper would be sufficient to germinate the spores of most fungi. *Fusarium solani* under those conditions would not invade the tissue of dasheens; but if they were sprayed twice a day for one or two days so that the spores would be suspended in a film of water germination and invasion of the tissue would take place before an impenetrable corky layer had formed over the wound. Some root crops have the power to absorb a considerable quantity of water, so that even though water of condensation may be formed on the glass of a moist chamber, the specimen inside is comparatively dry. For example, five tubers of dasheens with a total weight of 558 gm. absorbed 21 gm. of water in 24 hours, or more than 3.7 per cent of their original weight; and ten sweet potatoes from storage with a total weight of 1,539 gm. absorbed 84 gm. in two hours, or nearly 5.5 per cent of their original weight. Both

dasheens and sweet potatoes continue to absorb water for some time, and sweet potatoes will take up as much as 7 to 20 per cent of their weight in 24 hours, depending naturally on how dry they were when immersed. Relatively the greatest absorption takes place during the first two hours; in extreme cases as much as 10 per cent. The rate of absorption drops off at the end of that time, but the curve continues steadily upward thereafter.

*Sclerotium rolfsii* also requires considerable moisture to start growth, but requires no addition of water to that in the filter paper of a moist chamber after 24 hours. This fungus may have the power after once becoming well established to penetrate the corky layer over a cut surface. Whether this is accomplished by the action of an enzyme was not determined.

*Diplodia tubericola* and the other closely related forms used in these experiments succeed better under exactly the opposite conditions. If the tubers after inoculation were subjected to the environment of the laboratory room, the results were better than if they were kept in a moist chamber. No attempt has been made to determine a cause for this phenomenon. It must be kept in mind that at the outset protection was afforded the spores and hyphæ by inserting them about a fourth of an inch into the tuber and the tissue squeezed together about the wound.

*Bacillus carotovorus*, like *S. rolfsii* and *F. solani* succeeded better if a film of moisture was sprayed on the cut surface for a day or two following inoculation. As soon, however, as decay set in, no further application was required, except to the filter paper in the bottom of the moist chamber. It should be noted in this connection also that dasheens, turnips, and carrots differ very much in respect to the moisture actually required to stimulate decay. Dasheens are very dry and absorb moisture quickly and must be sprayed several times to start decay. Turnips and carrots, on the other hand, require but little added moisture, decay starting more promptly and progressing more rapidly.

#### TEMPERATURE AS A FACTOR IN PRODUCING ROT

Temperature and moisture, so far as their relation to storage rots are concerned, are so closely associated that one can hardly be discussed independently of the other.

It is obvious that decay will not occur at a temperature at which the organism will not grow even in the presence of sufficient moisture or in the absence of moisture with the proper temperature.

RESULTS WITH *DIPLODIA TUBERICOLA*.—A number of dasheens inoculated with *Diplodia tubericola* from sweet potatoes were divided into two lots, one of which was placed in an incubator with a temperature varying from 34° to 35° C. The other lot was placed in an ice box with a temperature ranging from 12.2° to 13.5°. At the higher temperature

(34° to 35°) the tubers were more than half-rotted at the end of nine days, and in four more days all but one were decayed throughout. At the end of 20 days *D. tuberculosa* was isolated in pure culture from each. At a lower temperature (12.2° to 13.5°) all the tubers but one were perfectly sound at the end of 45 days. One tuber was half-decayed, and yielded *F. culmorum*.

The temperatures at which decay may be brought about by *S. rolfsii*, *F. solani*, and *B. carotovorus* were determined by the use of raw blocks of dasheen. After discarding the blocks contaminated in their preparation, the remainder were divided into two lots, one of which was inoculated with *S. rolfsii* and the other with *F. solani*. Each of these lots was divided into 6 groups of 10 tubers each and placed in different chambers of the Altman thermostat and in the laboratory room, the temperatures of which ranged as follows:

Chamber No.	Range of temperature.	Average temperature.	Chamber No.	Range of temperature.	Average temperature.
	°C.	°C.		°C.	°C.
5.....	8.2 to 10.0	9.1	Room.....	22.0 to 24.0	22.4
6.....	12.0 to 15.0	14.0	18.....	26.3 to 29.6	28.6
9.....	17.5 to 19.5	18.4	19.....	34.5 to 36.0	35.3

RESULTS WITH *FUSARIUM SOLANI*.—In all the chambers except No. 5 (9.1° C.) growth started in two days. While there was some difference in the general appearance of the growth in the different chambers, there was nothing strikingly characteristic. At the lower temperatures there was a slight reduction of hyphal growth compared with higher temperatures, accompanied by the production of a salmon-orange color on the blocks. At the higher temperatures, particularly in chambers 18 (28.6°) and 19 (35.3°), abundant hyphæ were produced. An accident to chambers 18 and 19 at the end of 10 days terminated that part of the experiment, but an examination of the tubes showed that the blocks were completely decayed and typical spores of the causal organism produced. The others were continued for 20 days longer. At the end of that time no decay had taken place in chamber 5 (9.1°), and no spores were formed, though a slight discoloration of the blocks had taken place. In all the other chambers the blocks were completely softened. In the tubes exposed to room temperature (22.4°) typical spores were produced, while in chamber 6 (14.0°) there were a few abnormal spores, in No. 9 (18.4°) many. In general it may be stated that while decay was complete in all chambers except in No. 5 (9.1°) spore production was better at the three higher temperatures. The results therefore seem to indicate that tubers stored at the higher temperatures are more liable to be decayed by *F. solani* than if stored at a temperature of 8° to 10° or lower.



RESULTS WITH *SCLEROTIUM ROLFSSII*.—This organism produced no decay at the end of 38 days in chamber 5 (9.1° C.), but a few immature sclerotia were formed. In all the other chambers visible growth appeared in two days. In chambers 6 (14.0°) and 9 (18.4°) hyphæ were abundantly produced and the sterile blocks completely decayed, but no sclerotia were produced at the conclusion of the experiments. At the three higher temperatures the blocks were also decayed, but the production of hyphæ was markedly less and the number of sclerotia relatively larger, increasing in number with the increase in temperature. It therefore appears that the minimum temperature at which this organism will produce decay is near 8° to 10°. Other things being eliminated, dasheens would apparently, from the results of these experiments, keep better if stored at a temperature of about 8° to 10°.

RESULTS WITH *BACILLUS CAROTOVORUS*.—Experiments to determine the range of temperature of *B. carotovorus* were made some months later by inoculating sterile raw blocks of turnips and dasheens. The blocks were inoculated with a 24-hour-old culture of strain 3616 grown in beef bouillon and exposed in a series of chambers of the Altman thermostat and in the laboratory room to the following average temperatures:

Chamber No.	Range of temperature.	Average temperature.	Chamber No.	Range of temperature.	Average temperature.
	°C.	°C.		°C.	°C.
2.....	3.5 to 5.0	4.0	9.....	15.7 to 20.0	17.3
3.....	5.2 to 7.0	6.1	Room.....	20.0 to 25.0	23.0
5.....	9.0 to 12.0	10.0	18.....	29.2 to 36.2	32.7
6.....	11.0 to 14.8	12.3	19.....	34.7 to 37.9	35.8
8.....	14.6 to 18.5	16.0	20.....	38.0 to 41.6	39.3

The tubes were kept in the chambers for 27 days. At the end of that time no growth had taken place in chamber 2 (4° C.) and but a slight growth in 3 (6.1°), and 20 (39.3°). In six days a slight decay had started in chamber 5 (10°) and in three days in chamber 6 (12.3°). At the end of 14 days the dasheens were completely decayed in chamber 5. The turnips, on the other hand, were only partially decayed at the close of the experiment. In chamber 6 the dasheens were completely decayed at the end of 11 days and the turnips nearly so at the end of 14 days. In all the other chambers decay was noticeable at the end of two days, but progressed more rapidly with the increase of temperature up to and including 18 (32.7°). At the end of 11 days the dasheens were completely decayed in chamber 8 (16°) and the turnips mostly so, while in 9 (17.3°) the dasheens were completely decayed in 7 days and the turnips in 11 days. In chamber 18 (32.7°) both dasheens and turnips were completely decayed in 3 days, while in 19 (35.8) decay was not complete until 10 days. A parallel series of tests was run

in the laboratory room ( $23^{\circ}$ ), and decay was completed in 10 days. From these results there is a wide range of temperatures at which decay by this organism will take place. It is apparent, however, that the optimum lies somewhere between  $32^{\circ}$  to  $35^{\circ}$  and the minimum at approximately  $4^{\circ}$ . The maximum temperature was not determined, but in view of the fact that a slight decay of the blocks occurred in chamber 20 ( $39.3^{\circ}$ ), it must be somewhat higher.

It is interesting to note in this connection that the dasheens in most of the chambers were more promptly decayed in this experiment than the turnips. In other experiments of a similar nature both in the laboratory room and in the thermostat chambers this has not always been the case. In fact, it has frequently happened that the turnips, and carrots also when they are included in the tests, were more promptly decayed than the dasheens. While a strain (3616) originally obtained from dasheens was used for inoculating the blocks, turnips and carrots inoculated with this strain in moist chambers were generally more speedily decayed than dasheens. While no positive explanation of such a condition will be attempted, it has been apparent throughout the whole course of the work that the condition of the material when used plays no little part in the results to be obtained. It has been noticed that fresh turnips and carrots decay after inoculation more readily than those that have been kept in the ice box or elsewhere under conditions permitting the escape of moisture and eventual withering. Dasheens, on the other hand, lose moisture more slowly and remain suitable for such experiments a much longer time.

#### SUMMARY

(1) There are four storage rots of economic aroids: Java blackrot caused by *Diplodia tubericola*, *Diplodia macluræ*, *Diplodia gossypina*, and *Diplodia* sp. from *Mangifera indica*; powdery grayrot caused by *Fusarium solani*; sclerotium-rot caused by *Sclerotium rolfsii*; and softrot caused by *Bacillus carotovorus*.

(2) All of the species of *Diplodia* cause a rot identical in character.

(3) All the causal organisms are wound parasites.

(4) The parasitism of each organism has been established by inoculation experiments.

(5) *F. solani* from the Irish potato produces a rot identical with the rot produced by *F. solani* from the dasheen.

(6) Several other organisms were studied, none of which were found parasitic.

(7) The Java blackrot organism produced decay better under relatively dry conditions.

(8) It was necessary to apply sterile water once or twice to the tubers and corms after inoculation with *F. solani*, *S. rolfsii*, and *B. carotovorus*. After decay had started, no further application of water was required.

(9) High temperatures were more favorable to decay than low temperatures.

(10) *B. carotovorus* alone produced decay at an average temperature below 9° C.

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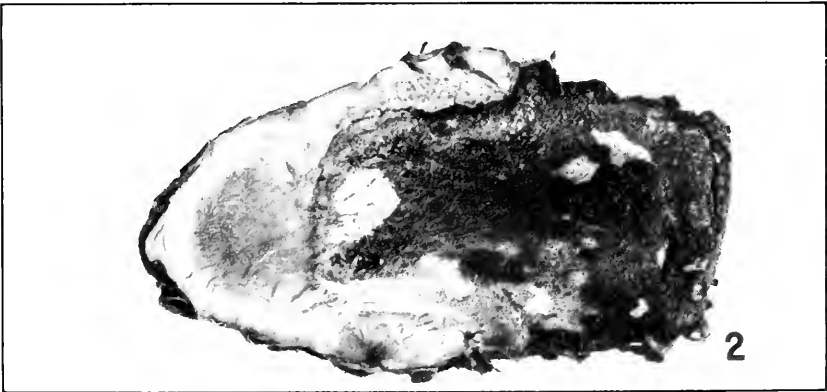
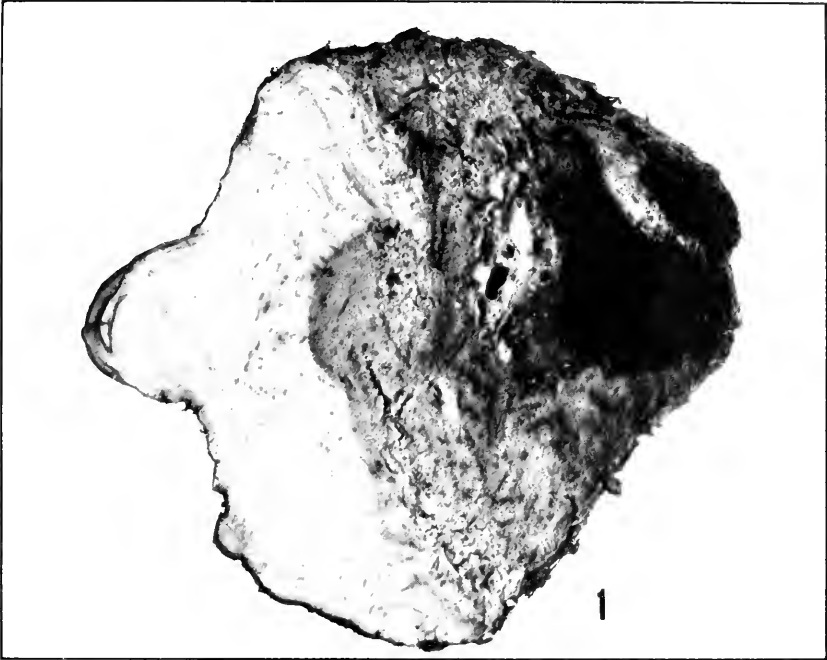
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PLATE LXXXI

Fig. 1.—A dasheen corm (*Colocasia esculenta*) showing Java blackrot produced by *Diplodia tubericola*. The blackrot end of the corm is separated from the healthy tissue by a dark brown area which in turn blackens later. Field material from Brooksville, Fla.

Fig. 2.—A corm of *Alocasia sp.* showing Java blackrot produced by *D. tubericola*. From a laboratory inoculation.

Fig. 3.—A dasheen tuber partially decayed by *Sclerotium rolfsii*. From a laboratory inoculation.



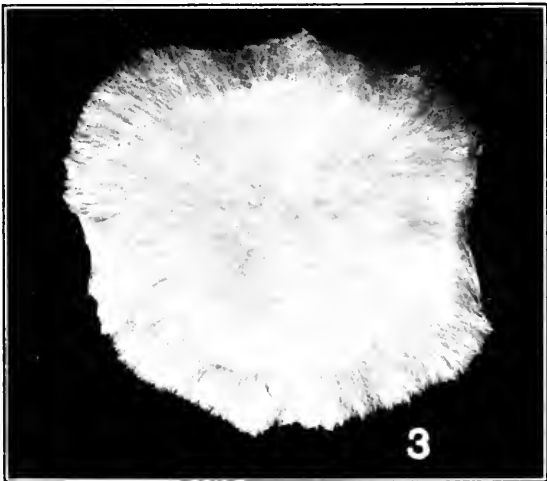
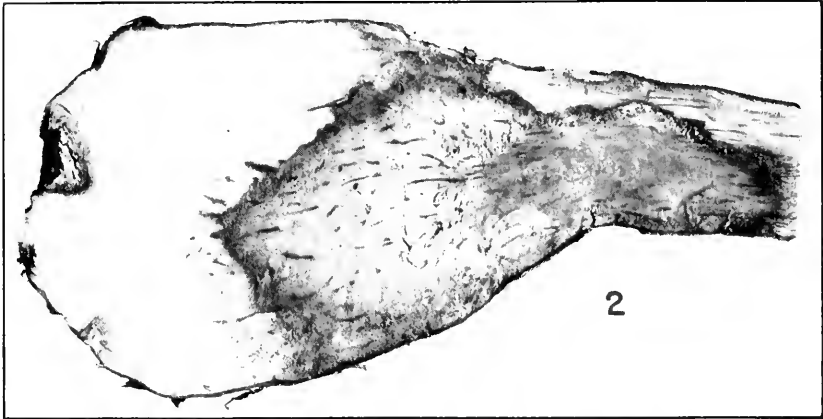
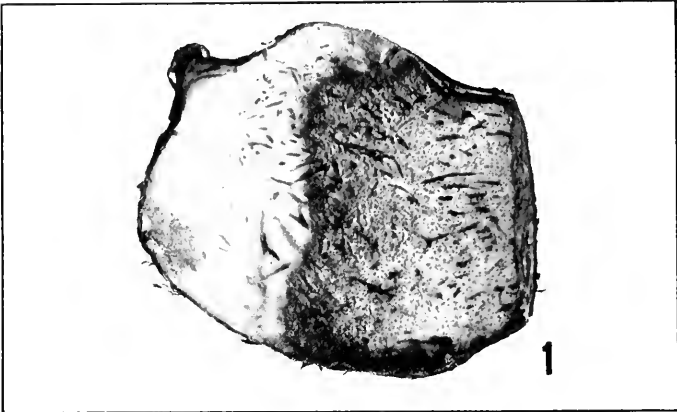


PLATE LXXXII

Fig. 1.—A tuber of *Colocasia esculenta* showing a powdery grayrot caused by *Fusarium solani*. From a laboratory inoculation.

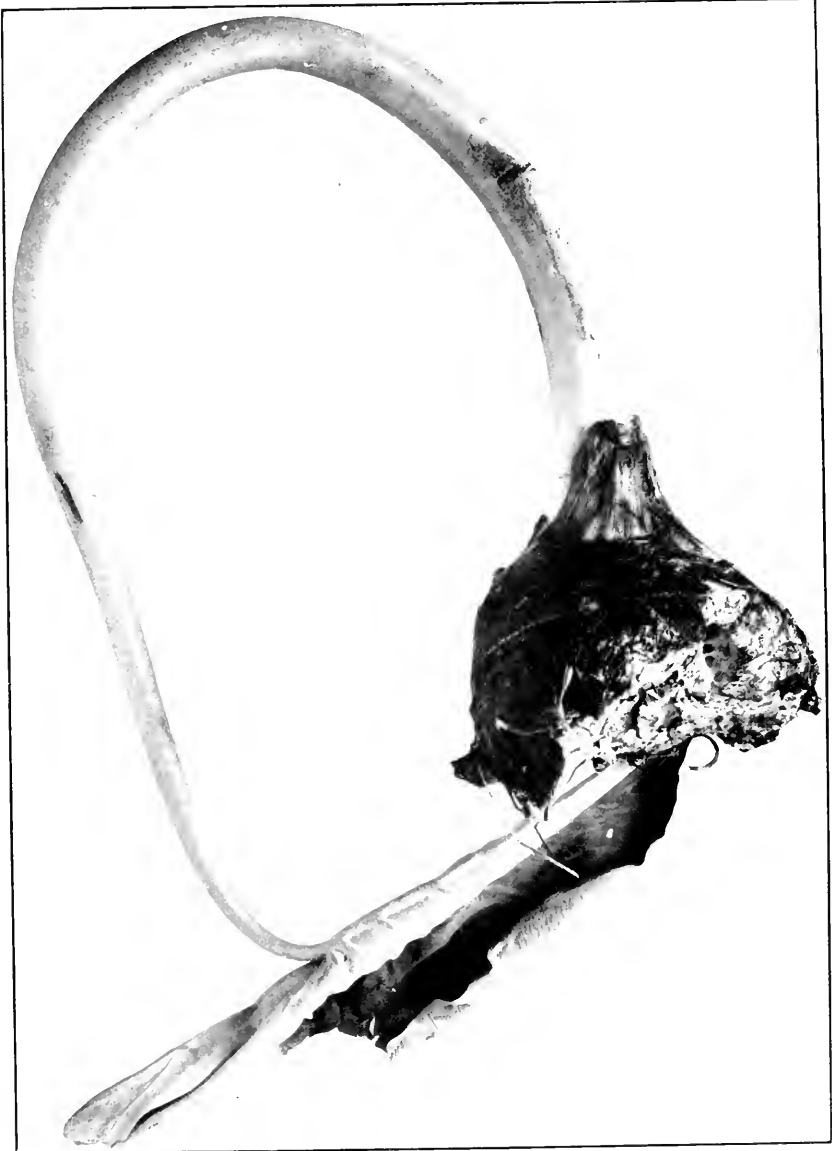
Fig. 2.—A tuber of *Xanthosoma sagittifolium* showing partial decay by *Fusarium solani*. From a laboratory inoculation.

Fig. 3.—A tuber of *C. esculenta* softened throughout by *Sclerotium rolfsii*. Note the hyphae over the entire surface. From a laboratory inoculation.

PLATE LXXXIII

A corm of *Colocasia esculenta* from Brooksville, Fla., mostly rotted away by *Bacillus carotovorus*. The organism isolated from this corm produced positive laboratory infections.







# EXPERIMENTS WITH CLEAN SEED POTATOES ON NEW LAND IN SOUTHERN IDAHO

[PRELIMINARY PAPER]

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It has generally been assumed by plant pathologists that if disease-free potatoes (*Solanum tuberosum*) were planted on new land the resulting product would be free from disease. For the past three years the writer has been engaged in investigations of potato diseases in southern Idaho, where this crop is grown under irrigation. As these irrigated tracts have but recently been opened up, there are many acres of land which may be classed as new in every sense of the word, since no agricultural crops have ever been grown upon them. Pathologists and potato growers alike believed that in these new lands just reclaimed from the desert lay a wonderful opportunity for the production of disease-free potatoes. However, from the beginning of the potato-growing industry in the irrigated portion of southern Idaho potato diseases have appeared each year. It is known that the first seed planted by the potato growers of these irrigated tracts was far from being free from disease, and it was naturally assumed that the diseases which appeared in the product had been introduced with the seed planted. The diseases most prevalent are wilt (*Fusarium oxysporum* Schlecht.), blackrot (*F. radicola* Wollenw.), jelly-end rot (*Fusarium* sp.), Rhizoctonia or russet scab, powdery dryrot (*F. trichothecioides* Wollenw.), and common scab.<sup>1</sup>

During the first two years of the author's investigations of potato diseases in southern Idaho, he observed that when potatoes were planted on virgin land just reclaimed from the desert many diseases usually appeared. Often the product from potatoes planted on such land appeared to be more diseased than that from potatoes planted on land which had been reclaimed from the desert for several years and which had been planted with other crops, such as alfalfa or grain. Frequently when such a diseased crop was observed, the grower would insist that the seed potatoes he had planted had been practically free from disease. Since certain of the diseases found, such as common scab and blackrot, are easily detected on the seed, the writer was forced to admit that in many such cases the grower might be right. Therefore,

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<sup>1</sup> No attempt has been made to isolate an organism from the common scab found in this region, but since its appearance is identical with that found in the East it is assumed that the causal organism is the same—namely, *Actinomyces chromogenus* Gasperini.

in the spring of 1915, experiments were set up to determine whether a clean product could be obtained by planting disease-free seed on new land. While these experiments are to be continued another year, the results of the first year's trials were so conclusive and of such importance to the potato-growing industry that it appears desirable to record them at the present time.

In the spring of 1915 arrangements were made with several farmers to plant clean seed on lands which had never before been planted to potatoes. The plots planted ranged from one-twentieth of an acre to 1 acre in size. Six of the plots were planted on virgin soil reclaimed from the desert for the express purpose of planting with disease-free seed potatoes. Fourteen of the plots were planted on land which had for several years been in alfalfa or grain. On the grounds of the experiment station at Jerome, Idaho, other plots were planted with disease-free seed.

The land at the experiment station was reclaimed from the desert in 1910, planted to barley, and thereafter to alfalfa.

The varieties planted in the test plots were as follows: Idaho Rural, Netteed Gem, Rural New Yorker, Pearl, Peoples, Red Peachblow, Burbank, Carmen No. 3, and Early Six Weeks. The disease-free seed was selected in the same manner for each plot as follows: Each tuber was first carefully examined for all external evidence of disease, such as common scab and the sclerotia of *Rhizoctonia* sp. All tubers showing evidence of either of these diseases were rejected. No tubers showing any large amount of infection with powdery dryrot were used. If there was only a small pocket of dryrot present, the infected portion was cut out until the tissues appeared white and clean. The externally clean tubers were then cut, the first cut being made across the stem end. The stem end portion was invariably discarded. If there was no evidence of vascular or other discoloration, the balance of the tuber was considered free from disease and was cut into pieces averaging about 2 ounces each. After cutting, the tubers were disinfected for 1½ hours in a solution of mercury bichlorid (1:1,000).

Throughout the season each plot was carefully watched, cultures being made from time to time as evidence of disease appeared in the plants. Wilt was found in every plot and *Fusarium oxysporum* was obtained in artificial cultures from stems showing vascular discoloration. Stem lesions and footrots were especially severe in all of the desert (or virgin) land plots. In all of the desert-land plots the plants presented a sickly appearance as compared with the plants in the alfalfa and grain land plots. There were indications in each of the desert-land plots of light yields and of a diseased product.

At harvest time the following methods were employed to determine the diseased condition of the tubers: In each of the smaller plots 100 hills were dug and the product of each hill examined separately. The

tubers were first examined for the presence of external diseases, such as Rhizoctonia or russet scab, common scab, blackrot, and jelly-end rot, after which each tuber was cut to determine the presence or absence of infection in the vascular tissue. The method employed in each of the larger plots was the same as in the smaller ones, except that several lots of 100 hills each were dug in different parts of each plot. All tubers showing pronounced vascular discoloration were considered as infected with wilt caused by *Fusarium* spp. Tubers showing such discoloration were taken to the experiment station laboratory and cultures were made from the discolored vascular tissue. Eighty per cent of all such cultures showed the presence of either *F. oxysporum* or *F. radicola*. The percentage of vascular infection present in the harvested product was estimated on this basis.

The average percentage of disease present in the alfalfa-grain land plots, planted with disease-free seed, including the plots at the Jerome experiment station, was as follows: Common scab, 4.7 per cent; Rhizoctonia or russet scab, less than 2.8 per cent; vascular infection, 26 per cent; and fieldrots caused by *Fusarium* spp., less than  $\frac{1}{2}$  of 1 per cent. In the desert-land plots the averages were as follows: Common scab, 9.3 per cent; Rhizoctonia or russet scab, 11.6 per cent; vascular infection, 29.3 per cent; and fieldrots caused by *Fusarium* spp., 5.6 per cent. The fieldrots caused by species of *Fusarium* are blackrot (*F. radicola*) and jelly-end rot, the causal organism of which has not been definitely determined, but with it are associated *F. radicola* and *F. oxysporum*, as well as other species of *Fusarium*. Of these two fieldrots, blackrot was the one principally found. Jelly-end rot was confined to the Netted Gems and rarely occurred.

It will be seen that the percentage of disease was much higher in the plots planted on virgin soil than in the plots planted on land which had previously been cropped with alfalfa or grain. When the fact is taken into consideration that the yield in each of the desert-land plots was light and the tubers small and of poor quality, it must be admitted that raw desert lands are not well adapted to the production of high-grade seed stock.

From the results so far obtained from the experiments the following conclusions are drawn:

(1) Planting clean seed potatoes on new land does not guarantee a disease-free product.

(2) A smaller percentage of disease may appear in the product when clean seed is planted on alfalfa or grain land than when similar seed is planted on virgin or raw desert land.

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# JOURNAL OF AGRICULTURAL RESEARCH

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# JOURNAL OF AGRICULTURAL RESEARCH

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## DIGESTIBILITY OF VERY YOUNG VEAL

By C. F. LANGWORTHY, *Chief*, and A. D. HOLMES, *Scientific Assistant*,  
*Office of Home Economics, States Relations Service*

### INTRODUCTION

Throughout the United States little was known until recently regarding very young veal, since the sale of calves less than 3 to 6 weeks old for food is prohibited by Federal and State laws. Our attitude toward veal, as toward many other foods, has been determined in part by custom and prejudice and in part by economic conditions and experience, often being illogical; therefore it is of interest to ascertain in such cases how far belief is justified by facts, as shown by controlled experimental tests.

That the common opinion that veal is less wholesome than beef and young veal less so than mature veal is not a consistent prejudice against young flesh foods is shown by the common and apparently growing taste which prefers squab to pigeon, ranks broilers as superior to fowls, considers sucking pig a great delicacy, and regards hothouse lamb—that is, lamb less than 3 months old and rapidly grown and fattened—as much superior to older lamb as lamb is to mutton.

That economic conditions may have an effect upon opinion, which is not consistent, is shown by the situation with respect to the marketing of calves. In grazing areas where the cheapness of food makes it possible to rear cattle at least to early maturity there is a natural tendency to do so. In regions where the dairy industry is highly developed, milk is such an important product that it is not thought profitable to rear calves beyond the period when the mother's milk becomes salable, and so, even though they can not be marketed and the producer will seldom care to use on his own table what he is prohibited from selling, they are often slaughtered at from 3 to 6 days old instead of fed until the lawful marketable age.

The prejudice against veal, and more particularly young veal, is inspired chiefly, it would seem, by the belief that it is indigestible, by which is meant either that it causes a digestive disturbance or that it

fails to digest as thoroughly as other meats, with the result that it is either harmful or undesirable as food. That this opinion does not rest on general experience is indicated by the contrary belief in Europe, which ranks veal as a particularly desirable meat, even for invalids, and which regards very young veal much as it does young pig and young lamb.

The question of its dietary value and its digestibility, both in the more popular as well as in the technical sense, thus becomes one worthy of study for itself and for its bearing upon the common prejudice against the use of young veal as well as upon the related matter of wholesomeness when this food is eaten in comparison with more commonly accepted foods, and accordingly the tests here reported were undertaken.

#### PREVIOUS EXPERIMENTS

In the literature consulted empirical conclusions are not uniform and very little definite information has been found regarding the food value and possible use in the diet of very young veal.

Studies have been made, however, to determine what the difference is, chemically or otherwise, between very young veal and the older market veal. Fish (4),<sup>1</sup> for instance, conducted such an investigation, with the object of obtaining data which would enable him to determine the relative age of the animal in market, so that the very young could be detected. He determined the specific gravity and freezing point of the tissue juice and also the percentage of water, finding that in very young veal, where more water is present in the tissues, there is less depression of the freezing point and a lower specific gravity. In continuation of this work, the same author (5) made dietary studies to determine whether the flesh of the young calf from 1 to 14 days of age exerts any injurious effects upon the consumer. Seven families, including over 20 individuals from 2 to 60 years of age, ate this meat and reported no physiological disturbances, the health of each remaining apparently normal. Later, the results of experiments *in vitro* led Fish (6) to conclude that the difference in the thoroughness of digestibility of the tissues of very young and market veal is so small as to be practically negligible.

Very recently Berg (3) has reported the experimental data of a biochemical comparison of beef and immature veal in which he discusses the chemical composition, digestion *in vitro*, and results of feeding experiments made with animals (cats) in the laboratory. He concludes that there are no physiologically significant differences in the chemical composition of beef and immature veal. In a large number of artificial digestion experiments he found that immature veal was as quickly digested as beef. In the feeding experiments with cats, immature veal supplied all of the nitrogen and a large share of the energy of the

<sup>1</sup> Reference is made by number to "Literature cited," p. 587-588.

diet. The animals remained in a normal condition at all times, exhibiting characteristic functions of growth, maintenance, and reproduction. Berg reports that the immature veal at times was kept in an ice box at 2° to 4° C. for many days before use and that it remained in edible condition.

It is evidently the opinion of the more recent investigators that very young veal, or, as it is commonly called, bob veal, is not unsuited for use as human food. As very little information, however, is available as regards the coefficient of digestibility of very young veal, a series of experiments was undertaken to determine the completeness of digestion of this material by the human subject in normal health.

#### DIETARY TESTS

Before attempting to study the digestibility of very young veal, tests were made in which it was cooked in the laboratory and in several homes and eaten in quantity, although no record was kept of the amounts of veal and other foods eaten. The purpose of these tests was to have very young veal prepared by different methods and eaten by a large number of persons whose ages and daily activities were quite varied, to see whether purging or other disturbances of digestion would result and whether there was warrant for the popular belief that it is indigestible in the sense that it causes illness or distress. In general, it may be said that no physiological disturbances were noted either in the laboratory tests with individuals or in the tests made in a number of families.

Reports of the individual tests of the use of very young veal are as follows:

In family A the ages of the various members ranged from 4 to 65 years. Observations of the dietary value of bob veal were made at various times, using different portions of the carcass. With one exception no member of the family was apprised of the age of the veal, which was cut up into small pieces and prepared in the form of a stew. The criticism offered in regard to the meat was that it seemed somewhat dry, and one member of the family remarked that it seemed to be stringy. No one experienced any ill effects whatever from eating the meat, and all appeared to relish it as much as market veal.

Family B was comprised of comparatively young adults only. All the members of the family were apprised of the nature of the meat, which was served in the form of a roast. Their criticism of the veal was that it seemed rather tasteless—that is, lacked flavor—and that although it appeared good it would not be preferred to the ordinary market veal. No instances of any ill effects resulted from eating this meat.

Family C was composed of both children and adults whose ages ranged over a period of 50 or more years. In this study tests were made at two different times. On one occasion the veal was served in the form of a stew, while in the other case it was served roasted. Only one member

of the family knew the age of the veal. All appeared to enjoy the meat and made no remarks which would indicate that they were aware of its nature. No physiological disturbances were noted during these tests.

In general, it was noticed that when bob veal was cooked as a roast it presented a less appetizing appearance than did the more mature meats. This is due principally to the greater amount of water in the meat and to the less firm structure of the muscular tissue; consequently, when the meat is roasted or broiled—methods of cooking which cause the evaporation of considerable of the water—the meat shrinks away from the bones, producing an abnormal and undesirable appearance. However, if the veal is removed from the bone, it may be roasted, broiled, or used to make stews with very satisfactory results.

The younger veal was found to take the place quite satisfactorily of the common market veal. In practice, the shrinkage in cookery due to loss of water would mean the purchase of a larger quantity for the table if the same amount of meat is to be eaten. The deficiency in fat can be made up by adding fat in cookery. No study was made of the effects of handling upon market quality, or of the general question of legal regulation with respect to the marketing of young veal.

#### DIGESTION EXPERIMENTS

##### SELECTION AND COMPOSITION OF MATERIAL

The series of digestion experiments reported was made in this laboratory at the request of the Bureau of Animal Industry. The age of the calves used (in every case supplied by the Bureau of Animal Industry) was never more than five days, this age being arbitrarily selected in order to have as great a difference in maturity as possible between this type of veal and market veal. This was done so that any difference in the digestibility of the two types would be easier of detection should a difference exist. The calves used were procured without regard to breed or size and were healthy individuals passed by the Federal meat inspectors.

The calf was slaughtered the day preceding the cooking of the meat, and the carcass was stored in the meantime in a well-cooled refrigerator, no attempt being made, however, to study the keeping quality of the meat under ordinary trade and household conditions, a matter which apparently has not been studied. The cut most generally used in the digestion experiments was the leg, while the remainder was used for the dietary studies. This cut was chosen since it was easy to obtain the same cut of market veal for check experiments. The waste material (bone, tendon, etc.) in the legs of the very young veal was determined and found to be approximately 40 per cent. This amount of waste is much greater than that of mature veal, which is reported as 12 per cent (maximum, 25 per cent; 1, p. 31-32). Since muscular tissue is less developed in younger animals, it is logical to expect that there will be less tissue in proportion to bone.

In order to make a comparison of the percentages of the principal food constituents in the two types of uncooked meat, an analysis of the very young veal and market veal is given below. The percentages which are reported here are those obtained by averaging the results obtained from the analysis of a number of different samples. Very young veal: Water, 76.09 per cent; protein, 18.48 per cent; fat, 2.79 per cent; ash, 0.99 per cent. Market veal: Water, 71.97 per cent; protein, 20.07 per cent; fat, 7.43 per cent; ash, 1.28 per cent. A comparison of the two types of veal shows that very young veal contained more water than market veal and correspondingly less protein and fat. However, when the meat was cooked, the difference in the amount of protein and fat in the two types of veal was lessened, owing to the loss of more water from the very young veal than from the market veal. It was found that there was an average of 33.23 per cent of protein in the former and 34.41 per cent in the latter type of veal, although it is obvious that these figures represent merely the protein content of meat cooked by a single method. Obviously, if the meat were cooked in another way, for a longer or shorter period of time, using a different amount of fat, or employing more or less heat, the composition would vary quite materially. The chief difference in the composition of the meat from the very young and the older calves is in the percentage of water present; this decreases as the animal grows older, while at the same time the percentage of fat in the meat increases. Aside from this, the meat of the two ages shows very little difference.

#### NATURE OF THE DIET

The very young veal was prepared by cutting in a meat cutter all the meat to be used for an experimental period. The meat was then thoroughly mixed to give a uniform product for eating and for analysis. After the meat had been prepared in this manner, it was cooked in the form of small cakes resembling Hamburg steak. A small amount of animal fat was used in cooking, but no attempt was made to increase materially the fat content of the meat cakes, and, roughly, the same amount was used for both bob veal and mature veal.

It has often been observed that the digestibility of a food is more satisfactorily determined if it be incorporated in a mixed diet than if eaten singly. Consequently, it was decided that the basal ration to be used in studying the digestibility of the meat in question should contain fruit, bread and butter, and tea or coffee with sugar, if desired. It can readily be seen that this diet contains all the essential constituents of a well-balanced ration, while at the same time the protein constituent of the diet is derived principally from the veal. It was impossible in these tests to prevent the subjects from knowing the nature of the diet. For instance, they all knew that they were having meat of some sort and that fat was used for the purpose of frying,

but it is hardly possible that they were definitely aware of the source of the meat. If this was the case, it is reasonable to believe that the appetite of the subjects and the digestibility of the food were not affected by any psychic factor.

The three-day or nine-meal experimental period, which is very often used in investigations of this kind, was again adjudged to be most satisfactory. In order that the subjects should experience no monotony while eating the ration, each test period of three days per week was followed by a rest period of four days; and, furthermore, the digestion experiments were conducted only on alternate weeks. During the intervening weeks tests were made of the digestibility of other food materials.

#### SUBJECTS

Five subjects assisted in making this investigation. They were active young men of good physique and, as dental students, all were sufficiently interested in physiological questions to appreciate the importance of carrying out carefully such directions as were given them. They were urged to observe accuracy especially in the collection of feces, since in considering the digestibility of any food material it is more essential to know the amount of food retained and assimilated by the body than only the total amount of food consumed. To assist in identifying the feces of the test period, charcoal, which imparts a dark color to the feces, was given with the first meal of the test period and with the first meal following the period. The feces showing a dark color and all excreted until the dark color imparted by the charcoal was again noticed were retained for analytical purposes. The subjects were asked to bring notes describing their physical condition before, during, and after each test period. They all reported that with the exception of one or two colds they were in normal physical condition during the entire time that the investigation was in progress. Consequently, it has not been considered necessary to give in detail any of the individual reports which were received.

#### EXPERIMENTS WITH VERY YOUNG VEAL

Inasmuch as lean meat like very young veal consists almost wholly of water and protein, these experiments are concerned only with the digestibility of protein. One method of determining the digestibility of a single food of a mixed diet is to determine by digestion experiments with the basal ration alone the amount of undigested residue occurring from the accessory foods, and for which a corresponding correction may be applied to the digestibility of the total ration. A second method consists in estimating the digestibility of the basal ration. Since the digestibility of the protein of wheat flour, fruit, and butter have been accurately determined by previous investigators, satisfactory factors are available

for estimating the digestibility of the protein furnished by these foods. Accordingly, in this investigation it has been assumed that the protein of bread is 93.8 per cent (8, p. 33), that of butter 97 per cent (2, p. 104), and that of fruit 85 per cent (2, p. 104) available. The following equations illustrate the method of applying the above factors:

[Weight of protein in bread, butter, fruit] × [Percentage of undigested protein in each] = [Undigested protein from basal ration].

[Total undigested protein] - [Undigested protein from basal ration] = [Undigested protein from meat alone].

[(Meat protein consumed) - (Undigested protein from meat)] ÷ [Meat protein consumed] = [Estimated percentage digestibility of meat protein].

The results which have been obtained in the tests of the digestibility of very young veal are given in Table I:

TABLE I.—Data of digestion experiments with very young veal in a simple mixed diet

Item.	Weight.		Protein.	
	Gm.	Gm.	Gm.	Per cent.
Experiment No. 5 (subject W. A. D.):				
Meat.....	736.0	227.4		30.90
Bread.....	707.0	67.9		9.61
Butter.....	254.0	2.5		1.00
Fruit.....	1,467.0	4.3		.29
Total food consumed.....	3,164.0	302.1		
Feces.....	41.0	15.6		38.07
Amount utilized.....		286.5		
Digestibility of entire ration.....				94.80
Estimated digestibility of very young veal.....				95.30
Experiment No. 6 (subject E. D. J.):				
Meat.....	704.0	217.5		30.90
Bread.....	942.0	90.7		9.63
Butter.....	207.0	2.1		1.00
Fruit.....	1,377.0	4.0		.29
Sugar.....	202.0			
Total food consumed.....	3,432.0	314.3		
Feces.....	65.0	28.8		44.31
Amount utilized.....		285.5		
Digestibility of entire ration.....				90.80
Estimated digestibility of very young veal.....				89.70
Experiment No. 16 (subject J. H. K.):				
Meat.....	703.0	287.6		40.91
Bread.....	745.0	66.5		8.93
Butter.....	154.0	1.5		1.00
Fruit.....	1,418.0	3.1		.22
Sugar.....	310.0			
Total food consumed.....	3,330.0	358.7		

TABLE I.—Data of digestion experiments with very young veal in a simple mixed diet—  
Continued

Item.	Weight.		Protein.	
	Gm.	Gm.	Gm.	Per cent.
Experiment No. 16—Continued.				
Feces.....	45.0	21.6		48.50
Amount utilized.....		337.1		
Digestibility of entire ration.....				94.00
Estimated digestibility of very young veal.....				94.10
Experiment No. 17 (subject W. E. L.):				
Meat.....	706.0	289.1		40.95
Bread.....	816.0	72.8		8.92
Butter.....	129.0	1.3		1.00
Fruit.....	1,557.0	3.5		.22
Sugar.....	188.0			
Total food consumed.....	3,396.0	366.7		
Feces.....	44.0	18.9		42.94
Amount utilized.....		347.8		
Digestibility of entire ration.....				94.80
Estimated digestibility of very young veal.....				95.40
Experiment No. 27 (subject W. A. D.):				
Meat.....	699.0	202.3		28.94
Bread.....	859.0	76.8		8.94
Butter.....	133.0	1.3		1.00
Fruit.....	1,218.0	3.0		.25
Total food consumed.....	1,909.0	283.4		
Feces.....	56.0	11.4		20.37
Amount utilized.....		272.0		
Digestibility of entire ration.....				96.00
Estimated digestibility of very young veal.....				97.30
Experiment No. 28 (subject J. R. F.):				
Meat.....	752.0	217.6		28.94
Bread.....	717.0	64.1		8.94
Butter.....	206.0	2.1		1.00
Fruit.....	1,209.0	3.0		.25
Sugar.....	68.0			
Total food consumed.....	2,952.0	286.8		
Feces.....	58.0	23.3		40.22
Amount utilized.....		263.5		
Digestibility of entire ration.....				91.90
Estimated digestibility of very young veal.....				91.40
Experiment No. 29 (subject W. E. L.):				
Meat.....	684.0	197.9		28.94
Bread.....	683.0	61.1		8.94
Butter.....	127.0	1.3		1.00
Fruit.....	1,280.0	3.2		.25
Sugar.....	115.0			
Total food consumed.....	2,066.0	263.5		



TABLE I.—Data of digestion experiments with very young veal in a simple mixed diet—Continued

Item.	Weight.		Protein.	
	Gm.	Gm.	Gm.	Per cent.
Experiment No. 29—Continued.				
Feces.....	68.0	32.5		47.82
Amount utilized.....		231.0		.....
Digestibility of entire ration.....				87.70
Estimated digestibility of very young veal.....				85.80
Average food consumed per subject per day..	964.2	103.6		.....

SUMMARY OF EXPERIMENTS WITH VERY YOUNG VEAL

Experiment No.	Subject.	Digestibility of protein.	
		Total diet.	Veal alone.
		<i>Per cent.</i>	<i>Per cent.</i>
5.....	W. A. D.....	94.8	95.3
6.....	E. D. J.....	90.8	89.7
16.....	J. H. K.....	94.0	94.1
17.....	W. E. L.....	94.8	95.4
27.....	W. A. D.....	96.0	97.3
28.....	J. R. F.....	91.9	91.4
29.....	W. E. L.....	87.7	85.8
	Average.....	92.9	92.7

From the data recorded in these tables it may be calculated that an average of 237 gm. of meat, furnishing 78 gm. of protein, or 75 per cent of the total protein of the diet, was eaten daily. The five subjects completed the experiments in good condition and without having experienced any physiological disturbances. The average values of seven experiments for the digestibility of total protein and that of meat protein alone are practically identical, being for the former 92.9 per cent and for the latter 92.7 per cent. The values estimated for the digestibility of bob-veal protein in the different experiments are not consistently higher or lower than the determined values for the protein of the total diet. This irregularity is very likely due to the variation in the amounts of protein obtained from the different sources.

CHECK EXPERIMENTS

Tests of the digestibility of market veal, using the same basal ration and with the same subjects and method of cooking as for bob veal, were made in order to compare the digestibility of very young and market veal under identical conditions. For this purpose legs of veal from animals at least 4 weeks old were purchased in the open market. Although it was realized that market veal contained a larger percentage

of fat than bob veal, only the superficial fat was removed before cooking, no attempt being made to secure meat cakes from market veal of the same composition as those made of bob veal. The fat contained in the market veal comprised the minor portion of the total fat content of the diet, and, moreover, for the sake of comparison with bob veal, it was necessary to know only the digestibility of protein. The digestibility of the protein alone, therefore, has been studied in these experiments. This is reported for the entire ration and has been estimated for the protein of meat alone in the manner previously described.

The results of three experiments with three subjects are given in Table II.

TABLE II.—Data of digestion experiments with market veal in a simple mixed diet

Item.	Weight.		Protein.	
	Gm.	Gm.	Gm.	Per cent.
Experiment No. 18 (subject J. H. K.):				
Meat.....	629.0	219.0		34.81
Bread.....	569.0	53.0		9.31
Butter.....	178.0	1.8		1.00
Fruit.....	1,568.0	3.0		0.19
Total food consumed.....	2,944.0	276.8		
Feces.....	38.0	17.1		45.00
Amount utilized.....		259.7		
Digestibility of entire ration.....				93.80
Estimated digestibility of market veal.....				93.90
Experiment No. 19 (subject W. E. L.):				
Meat.....	689.0	239.8		34.81
Bread.....	725.0	67.5		9.31
Butter.....	158.0	1.6		1.00
Fruit.....	1,536.0	2.9		0.19
Sugar.....	156.0			
Total food consumed.....	3,264.0	311.8		
Feces.....	57.0	24.8		43.5
Amount utilized.....		287.0		
Digestibility of entire ration.....				92.00
Estimated digestibility of market veal.....				91.60
Experiment No. 22 (subject W. A. D.):				
Meat.....	653.0	277.9		42.56
Bread.....	801.0	59.6		7.44
Butter.....	92.0	0.9		1.00
Fruit.....	1,702.0	4.3		0.25
Total food consumed.....	3,248.0	342.7		
Feces.....	62.0	24.1		38.94
Amount utilized.....		318.6		
Digestibility of entire ration.....				93.00
Estimated digestibility of market veal.....				92.00
Average food consumed per subject per day..	1,050.7	103.4		

SUMMARY OF EXPERIMENTS WITH MARKET VEAL

Experiment No.	Subject.	Digestibility of protein.	
		Total diet.	Market veal alone.
18.....	J. H. K.....	<i>Per cent.</i> 93.8	<i>Per cent.</i> 93.9
19.....	W. E. L.....	92.0	91.6
22.....	W. A. D.....	93.0	92.9
	Average.....	92.9	92.8

The digestibility of the protein of the total diet was determined to be 92.9 per cent, while it was estimated that the protein of market veal alone was 92.8 per cent available, values somewhat lower than those found by Grindley (7) for roast veal and for meat in general. The amounts of food eaten in these experiments and those with bob veal were approximately the same and furnished the same average amount of total protein (103 gm. per day), indicating that both rations were eaten with equal relish.

CONCLUSIONS

As determined by the experiments herein reported, the digestibility of the protein of bob veal is the same as that found for market veal—namely, 93 per cent, in round numbers. The subjects of both dietary and digestion experiments, so far as could be learned, experienced no physiological disturbances during the experimental period or afterwards. The tests showed that such veal can be prepared for the table in palatable ways and that so far as could be judged it was not unwholesome when eaten in quantity. In the digestion experiments the average weight of protein supplied by the meat exceeded that generally furnished by the meat portion of the ordinary diet, indicating that very young or bob veal was not distasteful. The experiments here reported also indicate that the general opinion that young veal is a common cause of digestive disturbance or fails to digest as thoroughly as similar foods is not justified.

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# INFLUENCE OF CALCIUM AND MAGNESIUM COMPOUNDS ON PLANT GROWTH<sup>1</sup>

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## INTRODUCTION

Some investigators seem to question the advisability of using magnesium-bearing minerals in agricultural practices, since they deem magnesium detrimental to optimum plant growth. Magnesium in some forms is detrimental to plant growth. However, the natural carbonates, such as limestones and dolomites, are not detrimental but in reality beneficial to plant growth when applied in amounts sufficient to neutralize soil acidity. Plants were found to grow and mature normally in pure dolomite and limestone.

In scientific circles considerable attention has been paid to the theory that calcium and magnesium must occur in a definite ratio for the optimum production of crops. Loew claims to have proposed this theory in 1892 (15)<sup>3</sup>, and much work has been conducted along this line, especially during the last decade. From the data presented in the following pages it will be seen that the ratio, within wide limits, had no effects.

The presence of sufficient quantities of calcium and magnesium in all soils is essential for the profitable production of crops. Various forms and quantities of these two elements may largely control the yields and composition of the harvests.

It is a well-known fact that plants will tolerate larger amounts of an essential element than they require. The quantity of calcium and magnesium taken up by plants is dependent upon the amount available and upon the kind of plants. The silicates of calcium and magnesium are relatively insoluble, while the chlorids are very soluble. Dolomite is denser and less soluble than limestone but more soluble than magnesite. Synthetic compounds of magnesium are more soluble, however, than similar compounds of calcium.

Alfalfa, when grown in sand and soil cultures with varying amounts of calcium and magnesium minerals, such as dolomite and magnesite, also with prepared compounds of these two elements, such as the chlorids, sulphates, and carbonates, was found to contain varying amounts of

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<sup>3</sup> Bibliographic citations in parentheses refer to "Literature cited," p. 616-619.

calcium and magnesium. Some treatments showed as much as 52.5 pounds of calcium and 12.98 pounds of magnesium per ton of dry alfalfa. However, the above amounts were in excess of the absolute requirements, as smaller applications gave as large yields and the alfalfa contained only 28 pounds of calcium and 8 pounds of magnesium per ton of dry matter. On this basis 6 tons of alfalfa with a high-calcium content would contain 315 pounds of calcium and 77.88 pounds of magnesium, or the equivalent of 787.5 pounds of calcium carbonate and 272.5 pounds of magnesium carbonate. Wheat straw, when grown in pure dolomite, contained 14.48 pounds of calcium and 14.6 pounds of magnesium per ton, whereas when grown in the absence of excessive amounts of these two elements the straw contained only 5.96 pounds of calcium and 5.43 pounds of magnesium per ton.

#### REVIEW OF THE LITERATURE

Solution cultures and pot cultures have contributed largely to our present knowledge of plant nutrition. Woodward (42) found that the solid particles of the soil furnished nourishment to the growing plants and that water acted only as a carrier.

Wolf (4) found by using beans and maize in controlled solutions, that the concentration as well as the kind of salts in the solution effected plant growth. His results show that when the concentration of the external solution was more than 0.25 per cent it became the controlling factor; whereas if less than 0.25 per cent absorption was controlled by the solution within the roots.

Dassonville (1) found that cutinization and lignification of the epidermis of leaves occurred much more rapidly in distilled water than in nutrient solutions; also that the growth of hemp and buckwheat was not influenced by the presence or absence of calcium and magnesium.

The crop is the measure of the resultant of all factors. In accordance with the present knowledge any one or many of the factors can be controlled. Likewise, the total amounts of the elements essential for crop production can be quantitatively determined.

Magnesium is essential for the growth of any living cell. Calcium is likewise essential except for the lower fungi and lower algæ, which alone are able to exist without it. Loew (16, p. 44) shows that neutral oxalates are not poisonous to the lower fungi. He attributes the deleterious effects in higher plants to the change in the structure of the calcium-protein compounds, due to the formation of calcium oxalate, while the disturbance is brought about by the change in imbibition caused by the formation of potassium-protein compounds, and that magnesium may bring about this change provided there is a deficiency in calcium.

Reed (29) found calcium to be necessary to the activity and growth of chlorophyll-containing organs. Willstätter (40) has pursued in detail

the study of chlorophyll and finds it to be a magnesium compound with generally three times as much green pigment as yellow pigment. He found that the magnesium content of chlorophyll was constant in both land and sea plants; therefore, it must function other than as a catalyzer. Pfeffer (27, p. 425), Macdougall (17, p. 219), Peirce (26, p. 100) and others believe magnesium and calcium play an important and necessary function in plant synthesis and cell formation, but are unable to assign any specific rôle to either of these elements.

There has been considerable contention as to whether calcium could be replaced by other members of the group. Haselhoff (7) grew beans and maize in solutions containing varying quantities of calcium and strontium and concluded that strontium seemed to take the place of calcium, replacing it only when the supply of calcium was inadequate. But it must be remembered that he first used calcium and strontium together in the solution and later reduced the calcium. However, Loew (16, p. 48) was unable to substantiate these results when he used species of *Tradescantia*.

Loew explains the toxicity between calcium and magnesium as being due to the formation of an insoluble condition of the phosphoric acid being fixed by the calcium, and that the framework (15) of the nucleus and plastids is a double organic salt of calcium and magnesium. However, Meurer (19) and Nathansohn (23) offer another explanation: Cells being selective in their absorption of ions can check osmosis before a balance is reached between the solution within and without the cell, and the absorption of salts does not increase proportionally with the increase of concentration of the outside solution. Osterhout (24) using calcium nitrate and magnesium nitrate was unable to substantiate Loew's assertion.

Considerable work has been done upon the antagonism of respective salts for each other in solution. Kearney (11, p. 20) shows that calcium salts are most beneficial in reducing toxicity. Lipman (14) reports toxicity between magnesium and sodium but not between magnesium and calcium.

Numerous investigators have sought answers to the proposed theory of a lime-magnesia ratio with just as numerous and conflicting results. Solutions, pot cultures of soil and sand, and field soils have all been employed in attempts to settle the controversy. Ulbricht (34) showed that yellow lupines, barley, and vetch were injured by applications of lime, especially when it contained high percentages of magnesia. Magnesia apparently increased the proportional yield of grain in the case of barley and lupines. Dojarenko (2), however, concluded that the theory of a definite calcium-magnesium ratio was not tenable, as many Russian soils containing great excesses of calcium over magnesium were benefited by liming.

The results of water and soil cultures by Gössel (6) failed to substantiate the theory of a definite ratio of calcium to magnesium. He obtained the highest yields for beans and barley with water cultures when the ratio of lime to magnesia was 0.04 to 1, and concluded that the effect of liming is dependent upon the character of the soil and not upon a definite ratio of lime to magnesia. About this same time the Japanese investigators (22) were actively engaged with the problem. However, their results all seem to bear out the theory of a definite ratio.

Konovalov (12), a Russian investigator, reports studies with barley, millet, oats, and maize, varying the ratio of calcium oxid to magnesium oxid, as follows: 13.4 to 1, 6.7 to 1, 3.3 to 1, 0.8 to 1, and 0.4 to 1. He found that the yields tended to increase with the increase of lime application, provided the magnesia content remained constant. Notwithstanding these results, Voelcker (35, 36, 37) states that the ratio is best at 1 to 1.

Meyer (20, 21) found that with buckwheat and oats the dependence of maximum yields on a definite ratio of calcium to magnesium could not be proved even in the case of soils containing more calcium than magnesium or vice versa. Undoubtedly the most extensive investigations regarding a definite calcium-magnesium ratio have been conducted by Lemmerman (13) et al. They used six different soils and grew vetch, oats, barley, rye, wheat, clover, mustard, and buckwheat, with the investigations extending over three years, 1907 to 1909, inclusive. From the standpoint of yields the ratio had no effects within wide limits. Stewart (33) reports soils having 16.88 per cent of calcium oxid and 6.1 per cent of magnesium oxid which were cropped for 40 years without the addition of fertilizers, except in the case of sugar beets, which received manure. The 8-year average yields are 80 bushels for oats, 50.4 bushels for wheat, 262.3 bushels for potatoes, and 21.8 tons for sugar beets.

Wartiadi (38) used sand and water cultures with wheat and barley and found that calcium and magnesium were beneficial or detrimental in proportion to their relative amounts in the culture solution. Russell (30, p. 144) finds no connection between the lime-magnesia ratio and the productivity of the soil. Haselhoff (8) also failed to substantiate Loew's theory, while Hopkins (9, pp. 170-171) found magnesium carbonate beneficial up to 0.8 per cent when added alone and in connection with calcium sulphate in such amounts as to maintain a ratio of 4 to 7, respectively, of magnesium oxid and calcium oxid.

Gile (3) reports that with the chlorids of calcium and magnesium at low concentrations the ratio exerted no influence, while at high concentrations it was effective. Good yields of pineapples (4) were produced from soils in Porto Rico when the ratio varied between 1 to 13 and 73 to 1; and in one field where the ratio of calcium oxid to magnesium oxid was 1.461 to 1 a yield of 60 tons of sugar cane was realized.



Pisciotta (28), an Italian investigator, reports the analysis of 60 soils which show a wide variation in the lime-magnesia ratio, due to the variation in the lime content. Patterson (25) found that magnesian lime, which is claimed to be poisonous, gave the highest yields.

In summing up the literature studies previously mentioned, it will be seen that Loew and his associates and Japanese students maintain the theory of a definite lime-magnesia ratio, as do Ulbricht and Wartiadi, whereas Dojarenko, Gössel, Konovalov, Meyer, Lemmerman, Haselhoff, Gile, and Patterson claim that a definite ration of lime to magnesia is not tenable and, furthermore, lacks substantiation.

Lemmerman et al. have undoubtedly conducted the most extensive investigations upon this subject and conclude that there is no correlation between maximum crop productions and the ratio of lime to magnesia. Soils reported by Russell and by Gile show wide variations in the lime-magnesia ratio, also in the percentages of these two elements, and that there fails to be any correlation between the productivity of a soil and its ratio of lime to magnesia.

Solution cultures show that a specific ratio of lime to magnesia is not equally effective in dilute solutions and in concentrated solutions. This indicates that the effectiveness is dependent upon the total balance of all the salts in solution instead of merely the ratio of calcium to magnesium.

The preponderance of evidence appears to be against a definite ratio of lime to magnesia, especially with respect to soil cultures in pots and under field conditions. What really seems of first magnitude is the resultant of all factors—that is, the climate, the plant, and structure, reaction, micro-organic activity, and composition of the soil.

#### EXPERIMENTAL WORK

These experiments were planned with the idea of studying the effects of calcium and magnesium upon plant growth when applied in different natural and in artificially prepared forms. Studies were made to determine the amount of calcium and magnesium which the plants could tolerate. The relation between the ratios of these two elements in the plants, in the soils, and in the materials applied was also studied.

Dolomite, limestone, magnesite, calcareous soils, and brown silt loam were used as sources of the natural forms, while prepared materials, such as the carbonates, chlorids, and sulphates, served as sources of the artificial forms. Increasing amounts of the various forms were used, also a variance in the ratio of calcium to magnesium was employed. The earlier applications varied from 0.1 to 0.6 per cent of magnesium added in magnesium carbonate and in magnesite. Later the following amounts were employed: 2, 6, and 10 per cent of magnesium in magnesite; 10 and 12.7 per cent of magnesium in dolomite; 0.1, 0.01, and 0.001 per cent of magnesium in the carbonates, chlorids, and sulphates. In each series sand or soil was used as a control.

## DESCRIPTION OF PROCEDURE AND METHODS

Earthen pots 6.5 inches in diameter by 7.5 inches in depth were used. Each pot contained 13.2 pounds of sand, while in the soil series each contained 8.8 pounds of brown silt loam. Sand and soil were used as mediums of control, and to these two materials were added the various forms and amounts of calcium and magnesium.

Various methods were pursued in extracting the sand. At first dilute hydrochloric acid (HCl) was kept in contact with the sand for 48 hours, but this failed to remove all the calcium and magnesium. Later the sand was extracted with stronger acid (1,350 c. c. of concentrated hydrochloric acid plus 1,000 c. c. of distilled water) for periods of from 9 to 14 days. Sand was also digested on a steam bath for 4 days with this same strength acid. None of the above processes were able to remove all the calcium and magnesium from the sand, as will be seen from the analysis reported.

At intervals varying from 10 days to 2 weeks plant food was added from the following solutions:

(1) Potassium sulphate, 50 gm. to 2½ liters of water; (2) ammonium nitrate, 80 gm. to 2½ liters of water; (3) disodium phosphate, 26.1 gm. to 2½ liters of water; (4) ferric chlorid, 0.4 gm. to 1 liter of water.

The calcium and magnesium were applied in forms previously mentioned. The moisture content of the sand was at first 12 per cent, but it was later raised to 14 per cent, while for the brown silt loam it was 24 per cent. Every 10 days the pots were brought to standard weight by adding distilled water.

All crops were grown in the agronomy greenhouses at the University of Illinois. The principal crops used in these studies were wheat (*Triticum* spp.), alfalfa (*Medicago sativa*), soybeans (*Soja max*), and cowpeas (*Vigna sinensis*). Oats (*Avena sativa*), clover (*Trifolium pratense*), timothy (*Phleum pratense*), and sweet clover (*Melilotus alba*) were also used to test the effect of artificial carbonates upon germination.

In the wheat and soybean series, 10 seeds per pot were planted and 7 plants permitted to grow, while for alfalfa 15 plants were permitted to grow whenever possible.

In making determinations for calcium and magnesium, the soils were first fused with sodium peroxid and from this point the usual method was employed. The calcium oxalate was dissolved in dilute sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and the calcium calculated from the amount of *N/10* potassium permanganate required to oxidize the oxalic acid thus formed. The magnesium was precipitated as magnesium-ammonium phosphate and burned to the pyrophosphate. In analyzing the plants 2 gm. of finely ground material were ashed, taken up in hydrochloric acid, and the calcium and magnesium determined as above stated. Acid extractions of the dolomites and limestones proved as good as fusions.

TABLE I.—Composition of materials supplying calcium and magnesium to the soil

Material.	Calcium carbonate.	Magnesium carbonate.	Calcium.	Magnesium.	Molecular ratio of calcium to magnesium.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	
Dolomite C <sub>1</sub> .....	43.65	35.26	.....	.....	5:4.8
Dolomite C <sub>3</sub> .....	51.18	44.9	.....	.....	5:5.2
Magnesite C <sub>4</sub> .....	.....	98.37	.....	.....	.....
Limestone <sup>1</sup> .....	84.2	5.85	.....	.....	.....
Brown silt loam.....	.....	.....	0.305	0.35	5:9.6
Calcareous soil.....	.....	.....	5.58	2.64	5:3.8
Sand extracted with dilute hydrochloric acid for 48 hours.....	.....	.....	.....	.....	.....
Sand extracted with concentrated hydrochloric acid for four days on a steam bath.....	.....	.....	.0142	.016	.....
Sand not extracted.....	.....	.....	.0128	.0089	.....
			.017	.0148	.....

<sup>1</sup> From Columbia, Ill.

The brown silt loam used in series C and D was taken from the surface of sod land and had only the coarser roots removed. The calcareous soil was taken from a layer varying from 8 to 12 inches in thickness and 72 inches below the surface in the forestry near plot 719 of the north farm of the university. The coarser pebbles were removed before using. The analysis in Table I refers to the portion used in growing crops; the coarser pebbles show a higher content of calcium and magnesium, or 13.34 per cent of calcium and 5.97 per cent of magnesium.

The 6,000 gm. of sand, after being extracted with hydrochloric acid, contained for each pot from 768 to 852 mgm. of calcium and from 540 to 960 mgm. of magnesium. From the following tables it will be seen that the plants had the power to obtain considerable quantities of the apparently insoluble calcium and magnesium silicate, as they obtained quantities in excess of the amount added in the seeds, although they show lower contents than plants grown in an excess of these materials. The chemically pure magnesium carbonate gave an immediate alkaline reaction to phenolphthalein upon the addition of distilled water. Dolomites C<sub>1</sub> and C<sub>3</sub> likewise showed an alkaline reaction to this indicator, but only after having been in distilled water over night. Magnesite after standing in distilled water from 8 to 12 hours was alkaline to phenolphthalein, as was also the calcareous soil.

In experimenting with artificially prepared magnesium carbonate, a great deal of care was taken to obtain the alkali-free substance to begin with. This can be prepared by a precipitation from solution with ammonium carbonate. Magnesium carbonate in the presence of water has a great tendency to hydrolyze, which may at least partially explain its poisonous effect.

EFFECT OF MAGNESIUM AND CALCIUM IN PREPARED CARBONATES AND IN DOLOMITE UPON WHEAT AND ALFALFA GROWN IN SAND (SERIES A AND B)

The sand used in series A and B was extracted as previously described, washed free from acid, and the application made on the moisture-free basis. Pots 1 and 2 of series A and pots 21 and 22 of series B received no calcium or magnesium. Pots 3 and 4 of series A and pots 23 and 24 of series B received 2 per cent of dolomite  $Ca$  or 0.2 per cent of the element magnesium. Pots 9 to 20, inclusive, of series A and pots 29 to 40, inclusive, of series B received magnesium and calcium in prepared carbonate in amounts varying from 0.1 per cent to 0.6 per cent of magnesium. The magnesium carbonate was alkaline and of the following formula:  $Mg(OH)_2 \cdot 4MgCO_3$ .

Table II contains the yields for the above treatments. When added to sand, the lowest applications of magnesium in the prepared carbonate very materially retarded germination and inhibited growth; whereas amounts of 0.6 per cent of magnesium in dolomite caused no injury and even benefited growth.

The ratio of calcium to magnesium throughout this and all succeeding tables is reported as molecular calcium to molecular magnesium, with calcium always expressed as 5. The yields and percentages in all the tables are reported on the water-free basis. The wheat, series A, was harvested 83 days after being planted, while the alfalfa, series B, was harvested 84 days after being planted.

TABLE II.—Yields of wheat and alfalfa (in grams per pot on a water-free basis) in sand—series A and B

Treatment.	Molecular ratio of calcium to magnesium.	Wheat, series A.			Alfalfa, series B.		
		Pot No.	Tops.	Roots.	Pot No.	Tops.	Roots.
None.....		1	20.9	22.2	21	5.16	6.42
None.....		2	18.5	23.2	22	3.12	2.58
Percentage of magnesium in dolomite $Ca$ :							
0.2.....	5:4.8	3	7.07	5.4	23	6.24	8.73
.2.....	5:4.8	4	23.0	18.8	24	7.84	12.47
.4.....	5:4.8	5	23.0	15.0	25	5.97	9.0
.4.....	5:4.8	6	17.8	10.0	26	5.61	8.02
.6.....	5:4.8	7	20.7	12.9	27	7.75	10.34
.6.....	5:4.8	8	10.8	6.3	28	7.21	9.0

The pots receiving magnesium and calcium in the prepared carbonates in a ratio of calcium to magnesium, as 5 to 4, inhibited germination and permitted no growth.

Table III shows the analysis of the plants reported in Table II. The results here reported are the average of four determinations from duplicate pots.

From Table III it can be seen that the alfalfa is a heavier feeder on calcium and magnesium than is wheat, and that the percentages and the total amounts removed by the plants tend to increase with the increase in application, except where the calcium and magnesium are applied in the artificially prepared carbonates, in which case the lowest application is sufficient to inhibit growth and retard germination.

Throughout all the series the general tendency was for the calcium and magnesium content of the plants to increase with the increase in application. Wheeler (39) found that when magnesium was applied in the form of the sulphate the crop showed the ratio of magnesium oxid to calcium oxid to be as 1 to 1.13, but when magnesium was not present in the fertilizer the ratio of magnesium oxid to calcium oxid was 1 to 2.7.

TABLE III.—Analyses of wheat and alfalfa—series A and B

Pot No.	Substance added.		Composition of plants.				
	Calcium.	Magnesium.	Calcium.		Magnesium.		Molecular ratio of calcium to magnesium.
			Mgm.	Per cent.	Mgm.	Per cent.	
WHEAT STRAW							
1 and 2 <sup>a</sup> .....	0.0142	0.0165	37.0	0.187	21.4	0.108	5:5.1
3.....	.333	.2	68.5	.298	62.6	.271	5:7.6
7 and 8.....	.999	.6	60.1	.386	66.6	.421	5:9.1
WHEAT ROOTS							
1 and 2.....	.0142	.0165	43.4	.187	24.8	.09	5:4.1
3.....	.333	.2	85.3	.452	55.3	.292	5:5.4
7 and 8.....	.999	.6	91.8	.90	47.9	.50	5:4.35
ALFALFA HAY							
21 and 22 <sup>a</sup> .....	.0142	.0165	14.4	.347	6.84	.164	5:3.9
23 and 24.....	.333	.2	181.0	2.505	30.6	.431	5:1.4
27 and 28.....	.999	.6	196.5	2.622	48.6	.649	5:2
ALFALFA ROOTS							
21 and 22.....	.0142	.0165	8.52	.19	6.26	.14	5:6.1
23 and 24.....	.333	.2	48.8	.40	50.0	.472	5:8.5
27 and 28.....	.999	.6	54.4	.561	78.9	.815	5:12

<sup>a</sup> Pots 1, 2, 21, and 22 were extracted sand and received no calcium and magnesium.

EFFECTS OF CALCIUM AND MAGNESIUM IN PREPARED CARBONATES AND IN DOLOMITE UPON WHEAT AND ALFALFA IN BROWN SILT LOAM (SERIES C AND D)

Magnesium and calcium in prepared carbonates were less harmful in brown silt loam than in sand (series C and D). In fact, applications of 0.1 per cent of magnesium or 0.35 per cent of magnesium carbonate gave an increase over the check, and 0.7 per cent of the carbonate was practically as good. It must be remembered that the soil before treatment contained 0.305 per cent of calcium and 0.352 per cent of magnesium. The calcium and magnesium were added in the relation of 5 to 4, but the amounts in the soil changed this ratio to 5 to 7.1. Applications of 3.5 tons of prepared magnesium carbonate per acre were beneficial, 7 tons were about equal to the check, while upward of 10 tons caused practically no growth of the plants.

TABLE IV.—Yields of wheat and alfalfa (in grams per pot on a water-free basis) in brown silt loam—series C and D

Treatment.	Molecular ratio of calcium to magnesium.	Wheat, series C.			Alfalfa, series D.		
		Pot No.	Tops.	Roots.	Pot No.	Tops.	Roots.
Brown silt loam only.....	5:9.6	41	8.9	6.8	65	4.64	6.86
Do.....	5:9.6	42	9.3	7.9	66	4.28	6.53
Percentage of magnesium in magnesium carbonate plus calcium carbonate:							
0.1.....	{ 5:7.1	43	11.6	7.16	67	5.96	6.95
	{ 5:7.1	44	11.0	7.0	68	5.35	6.5
.2.....	{ 5:7.1	45	8.1	5.55	69	2.58	2.49
	{ 5:7.1	46	8.1	6.07	70	3.03	2.85
.3.....	{ 5:7.1	47	1.45	1.81	71	.09	.....
	{ 5:7.1	48	4.08	3.17	72	2.58	1.69
.4.....	{ 5:7.1	49	.9	.72	73	.....	.....
	{ 5:7.1	50	.18	.18	74	.....	.....
.5.....	{ 5:7.1	51	1.85	2.35	75	.....	.....
	{ 5:7.1	52	.09	.09	76	.....	.....
.6.....	{ 5:7.1	53	.09	.09	77	.....	.....
	{ 5:7.1	54	.09	.....	78	.....	.....
Percentage of magnesium in dolomite Ca:							
0.2.....	{ 5:4.8	55	9.52	5.45	79	5.17	6.05
	{ 5:4.8	56	10.5	6.07	80	5.0	5.08
.4.....	{ 5:4.8	57	9.61	8.15	81	5.35	6.85
	{ 5:4.8	58	9.07	7.25	82	5.08	5.88
.6.....	{ 5:4.8	59	10.0	8.8	83	4.37	3.74
	{ 5:4.8	60	9.52	8.15	84	5.43	6.15
2.....	{ 5:4.8	61	8.9	4.53	85	5.43	4.72
	{ 5:4.8	62	5.71	4.62	86	5.60	4.64
4.....	{ 5:4.8	63	6.07	4.53	87	5.17	5.08
	{ 5:4.8	64	7.52	4.53	88	5.52	3.65

MacIntire (18), while working with three different kinds of soils, found that 8 tons per acre of precipitated magnesium carbonate were decidedly toxic to wheat. He also found that both the oxides and the carbonates of precipitated magnesium were many times more soluble than the corresponding forms of calcium, while in the case of the native mineral

carbonates limestone was 1.62 times as soluble as dolomite and more than 3 times as soluble as magnesite.

Applications of dolomite Cr up to 40 per cent caused no injury to either wheat or alfalfa.

Table IV shows the treatments and yields of series C and D. Analyses of these plants are reported in Table V. Series C (wheat) was harvested 83 days after planting and series D (alfalfa) was harvested 84 days after planting.

From Table V it can be seen that wheat grown in soil (pots 41 and 42) shows 0.279 per cent of calcium and 0.256 per cent of magnesium, but when grown in extracted sand (Table III, pots 1 and 2) it had only 0.187 per cent of calcium and 0.108 per cent of magnesium, showing that there is a decided tolerance for these two elements. By comparing the wheat with the alfalfa it can be seen that, while alfalfa is a heavier feeder than wheat on calcium and magnesium, the proportional amounts of calcium are greater in alfalfa than in wheat.

TABLE V.—*Analysis of wheat and alfalfa grown in brown silt loam—series C and D*

WHEAT STRAW							
Pot No.	Substance added.		Composition of plants.				
	Calcium.	Mag- nesium.	Calcium.		Magnesium.		Molecular ratio of calcium to mag- nesium.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	
41 and 42.....	0.305	0.352	27.1	0.297	23.31	0.256	5:7.2
43 and 44.....	.527	.458	33.6	.297	52.7	.467	5:13.1
47 and 48.....	.971	.671	9.76	.352	32.8	1.19	5:28.1
55 and 56.....	.315	.2	35.4	.353	33.8	.337	5:7.9
63 and 64.....	6.30	4.0	29.2	.43	31.0	.456	5:8.8
WHEAT ROOTS							
41 and 42.....	.305	.352	21.9	.298	19.6	.266	5:7.5
43 and 44.....	.527	.458	29.8	.419	25.1	.352	5:7
47 and 48.....	.971	.671	9.7	.352	15.5	.626	5:14.1
55 and 56.....	.315	.2	34.0	.53	22.8	.397	5:6
63 and 64.....	6.3	4.0	50.0	1.102	34.1	.752	5:5.7
ALFALFA HAY							
65 and 66.....	.305	.352	71.0	1.595	17.7	.398	5:2.2
67 and 68.....	.527	.458	78.4	1.56	35.3	.624	5:3.8
71 and 72.....	.971	.671	11.4	.86	12.82	.96	5:9.3
79 and 80.....	.315	.2	85.5	1.684	25.0	.492	5:2.4
87 and 88.....	6.30	4.0	98.5	1.84	27.8	.521	5:2.3
ALFALFA ROOTS							
65 and 66.....	.305	.352	21.46	.326	34.0	.462	5:11.8
67 and 68.....	.527	.458	21.2	.314	37.5	.558	5:14.7
71 and 72.....	.971	.671	10.64	.628	17.2	1.00	5:13.3
79 and 80.....	.315	.2	25.0	.449	30.0	.54	5:10
87 and 88.....	6.30	4.0	96.0	2.2	59.2	1.358	5:5.1

## EFFECT OF MAGNESIUM AND CALCIUM IN DOLOMITE, MAGNESITE, AND PREPARED CARBONATES UPON WHEAT AND ALFALFA IN SAND (SERIES E AND F)

Table VI shows the yields of wheat and alfalfa when grown in sand and treated with increasing amounts of magnesium in magnesite. The applications vary between 0.1 per cent of magnesium and 0.6 per cent of magnesium or 0.35 to 2.1 per cent of magnesite. Chemically pure calcium carbonate was added to make a ratio of calcium to magnesium equal to 5 to 4. The wheat and alfalfa in these two series were grown to maturity, the seeds being ground up with the straw and an analysis of the composite made.

There was but little seed upon the alfalfa, owing to the fact that it was grown under screens and the fertilization was poor. However, the treatments seemed to cause no injury except where magnesium was applied in the form of the prepared carbonates in pots 104 and 120.

TABLE VI.—Yields of wheat and alfalfa (in grams per pot on a water-free basis) in magnesite—series E and F

Treatment.	Molecular ratio of calcium to magnesium.	Wheat, series E.		Alfalfa, series F.			
		Pot No.	Tops.	Pot No.	First crop, tops.	Second crop, tops.	Roots.
None.....		89	7.25	105	6.86	5.2	3.78
Percentage of magnesium in magnesite plus calcium carbonate:							
0.1.....	5:4	90	6.53	105	6.00	5.36	7.13
	5:4	91	7.16	107	8.35	5.8	5.46
.2.....	5:4	92	5.98	108	6.77	6.16	7.48
	5:4	93	9.88	109	6.25	4.94	4.57
.3.....	5:4	94	10.06	110	5.72	5.2	3.96
	5:4	95	6.8	111	5.9	5.1	4.32
.4.....	5:4	96	7.07	112	5.64	4.48	4.48
	5:4	97	7.8	113	6.77	5.02	1.85
.5.....	5:4	98	9.15	114	6.07	4.31	1.49
	5:4	99	7.8	115	7.92	4.4	2.38
.6.....	5:4	100	11.4	116	7.48	5.2	5.02
	5:4	101	6.25	117	7.32	5.55	3.52
Percentage of magnesium in dolomite C <sub>3</sub> :							
0.6.....	5:5.2	102	9.42	118	6.34	5.9	4.93
	5:5.2	103	10.86	119	5.72	4.66	2.64
Percentage of magnesium in magnesium carbonate.....	5:4	104	.....	120	.....	.....	.....

Table VII shows that the higher treatments have higher contents of calcium and magnesium in the plants. The wheat grown in sand (pot 89) shows only 3.3 pounds of calcium and 2.6 pounds of magnesium per ton, as against the pots treated with larger quantities of magnesite



(100 and 101), which show 5.52 pounds of calcium and 10.88 pounds of magnesium.

Gile and Ageton (5, p. 44) show that many plants such as soybeans, sugar cane, and sunflower have higher lime contents when grown upon calcareous soils and that the increase in lime content tends to decrease the amount of magnesia, iron, and potash.

TABLE VII.—Analysis of wheat and alfalfa grown in magnesite—series E and F

WHEAT STRAW							
Pot No.	Substance added.		Composition of plants.				Molecular ratio of calcium to magnesium.
	Calcium.	Magnesium.	Calcium.		Magnesium.		
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	
89.....	0.014	0.016	12.0	0.165	9.36	0.130	5: 6.5
90 and 91.....	.222	.100	10.55	.154	16.55	.241	5:13
100 and 101.....	1.332	.60	24.4	.276	48.1	.544	5:16.4
102 and 103.....	.967	.60	24.1	.237	32.3	.317	5:11.1

ALFALFA HAY							
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	
105.....	.014	.016	87.4	1.275	41.0	.598	5:9.3
106 and 107.....	.222	.100	106.8	1.49	45.1	.63	5:3.8
116 and 117.....	1.332	.60	98.5	1.345	56.0	.69	5:4.2
118 and 119.....	.967	.60	93.2	1.55	39.2	.653	5:3.5

ALFALFA ROOTS							
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	
105.....	.014	.016	25.4	.672	14.52	.385	5:4.8
106 and 107.....	.222	.10	55.7	.89	43.8	.64	5:6
116 and 117.....	1.332	.60	63.0	1.48	61.1	1.434	5:8
118 and 119.....	.967	.60	65.2	1.73	35.8	.95	5:4.6

EFFECT OF MAGNESIUM AND CALCIUM IN CALCAREOUS SOIL, MAGNESITE, DOLOMITE, AND PREPARED CARBONATES UPON WHEAT AND ALFALFA (SERIES G AND H)

It can be seen from Table VIII that the ratios of calcium to magnesium vary from one of 5 to 3.8 to one of 5 to 125 and that in both cases considerable growth occurred. However, in pots 135, 136, 161, and 162, receiving 35 per cent of magnesite and 100 gm. of calcium carbonate, the plants showed a yellow color and some sickness; still, in the case of alfalfa the plants were able to set some seed. The yields in pots 123, 124, 149, and 150, which received calcareous soil only, were somewhat less than where sand was mixed with the soil, owing to the soil being decidedly plastic and possessing a less desirable physical condition.

Plate LXXXIV shows the difference between some of the treatments in series G. Observe the small growth of the wheat in the pot receiving 6 per cent of magnesium in magnesite. This is due to the physical condition caused by applying the magnesite in a finely ground form, which caused a setting that resembled cement, whereas in the pot with 10 per cent of magnesium almost twice as much magnesite was applied, but in a coarser form. For the ratios in these pots see Table VIII.

Plate LXXXIV shows alfalfa growing under similar treatments.

TABLE VIII.—Yields of wheat and alfalfa (in grams per pot on water-free basis) in soil, dolomite, and magnesite—series G and H

Treatment.	Molecular ratio of calcium to magnesium.	Wheat, series G.			Alfalfa, series H.			
		Pot No.	Tops.	Roots.	First crop.	Second crop.	Third crop.	Pot No.
None.....	{.....	121	15.4	6.53	3.6	9.59	5.63	147
		122	1.08	.....	2.28	.....	.....	148
Percentage of magnesium in calcareous soil:								
2.64.....	{5:3.8	123	21.93	7.15	7.22	7.47	12.6	149
		124	21.0	14.7	7.04	7.22	10.02	150
1.328.....	{5:3.8	125	26.9	16.2	9.41	11.7	13.0	151
		126	27.3	10.4	9.59	12.35	10.9	152
0.672.....	{5:3.8	127	24.5	19.4	10.11	13.72	12.3	153
		128	28.62	9.25	10.02	12.3	9.59	154
0.344.....	{5:3.9	129	31.7	14.4	10.28	9.68	10.71	155
		130	32.6	10.32	9.68	10.46	7.83	156
Percentage of magnesium in magnesite plus calcium carbonate:								
2.0.....	{5:4	131	35.5	11.05	9.15	8.97	8.8	157
		132	35.8	10.05	10.9	9.86	8.88	158
6.0.....	{5:75	133	9.7	4.26	5.28	8.45	5.2	159
		134	30.8	1.18	5.9	10.62	3.69	160
10.0.....	{5:125	135	18.2	6.25	4.48	5.02	2.28	161
		136	17.4	6.16	10.11	9.77	9.59	162
Percentage of magnesium in dolomite Cr:								
12.7.....	{5:5.2	137	2.17	.....	5.2	9.77	9.77	163
		138	3.26	.....	.....	.....	.....	164

One of the most noticeable facts brought out in these series is the great sensitiveness of the plants to small quantities of calcium and magnesium, also their ability to utilize relatively insoluble forms of these two materials. In pots 121 and 122, Table IX, and pots 147 and 148, Table X, the plants were grown in extracted sand receiving no calcium and magnesium and were able to obtain considerable quantities that had not been removed by the acid extractions. The alfalfa was even able to mature a few seeds.

TABLE IX.—*Analysis of wheat grown in soil, dolomite, and magnesite—series G*

WHEAT STRAW

Pot No.	Substance added.		Composition of plants.				Molecular ratio of calcium to magnesium.
	Calcium.	Magnesium.	Calcium.		Magnesium.		
			<i>Mgm.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	
121.....	0.014	0.016	24.65	0.165	20.4	0.132	5:7
123 and 124.....	5.78	2.64	76.1	.358	52.9	.249	5:5.7
125 and 126.....	2.897	1.328	130.0	.48	78.7	.29	5:5
129 and 130.....	.732	.344	156.1	.485	97.5	.305	5:5.2
131 and 132.....	4.44	2.0	88.6	.248	188.0	.527	5:17.7
135 and 136.....	.666	10.0	66.8	.375	170.0	.955	5:21.2
137 and 138.....	20.47	12.7	15.6	.574	19.0	.73	5:12.7

WHEAT ROOTS

121.....	.014	.016	13.7	.287	8.14	.125	5:3.6
123 and 124.....	5.78	2.64	85.7	.795	35.6	.33	5:3.4
125 and 126.....	2.897	1.328	70.5	.584	44.3	.332	5:4.8
129 and 130.....	.732	.344	63.5	.513	30.7	.248	5:4
131 and 132.....	4.44	2.0	129.4	1.224	163.9	1.6	5:10.8
135 and 136.....	.666	10.0	22.25	.358	44.75	.72	5:16.8

The high percentage of magnesium in the plants grown in pots receiving 35 per cent of magnesite is also characteristic of tolerance. Likewise a high magnesium content tends to accompany plant sickness. In the case of wheat grown in dolomite, pots 137 and 138, there was a higher percentage of calcium than in any other treatment. A ton of water-free material contained 11.48 pounds of calcium and 14.6 pounds of magnesium, but a ton of dry matter from the treatment with 25 per cent of magnesite showed 7.5 pounds of calcium and 19.1 pounds of magnesium per ton, as against the check in sand which contained 3.3 pounds of calcium and 2.64 pounds of magnesium. Alfalfa tends to show the same thing, except that it is a decidedly heavier feeder upon these two elements than is the wheat crop.

The wheat, Table VIII, was planted on January 26, 1914, and harvested on May 27, 1914, making 121 days of growth. The alfalfa was also planted on the above date and the first crop harvested on May 27, 1914. The second crop was harvested 127 days later, on October 1, 1914, and the third crop on November 12, 1914, after 42 days of additional growth.

By comparing pot 147, Table X, for the three crops, it will be seen that the second crop of alfalfa contained practically three times as much calcium and magnesium per ton as did the first crop, while the time of growth was about the same. The third crop contained about twice as much calcium and magnesium per ton as did the first crop, and its period

of growth was only 42 days. This is due chiefly to the extensive development of roots, making it possible to utilize more of the small quantities of calcium and magnesium remaining in the extracted sand, for in the other pots where these two elements were added such striking differences do not occur in the different crops.

To each pot receiving 15 alfalfa seeds, 0.19 mgm. of calcium and 0.32 mgm. of magnesium were added in the seed, and for the three crops 164.5 mgm. of calcium and 90.72 mgm. of magnesium were removed. This indicates to what extent the plants may attack relatively insoluble compounds.

TABLE X.—Analysis of alfalfa grown in soil, dolomite, and magnesite—series H

## ALFALFA, FIRST CROP

Pot No.	Substance added.		Composition of plants.				Molecular ratio of calcium to magnesium.
	Calcium.	Magnesium.	Calcium.		Magnesium.		
			Mgm.	Per cent.	Mgm.	Per cent.	
147 and 148.....	0.014	0.016	11.2	0.382	5.72	0.198	5: 4.3
149 and 150.....	5.78	2.64	98.41	1.382	26.52	.373	5: 2.2
151 and 152.....	2.897	1.328	136.0	1.432	33.92	.357	5: 2
155 and 156.....	.732	.344	127.8	1.28	34.5	.346	5: 2.2
157 and 158.....	4.44	2.0	92.5	.922	74.5	.744	5: 6.8
159 and 160.....	.666	6.0	23.8	.426	52.3	.936	5:18.3
161 and 162.....	.666	10.0	76.7	1.05	83.7	1.148	5: 9.1
163.....	20.47	12.7	34.2	.66	33.8	.633	5: 8

## ALFALFA, SECOND CROP

147.....	.014	.016	105.3	1.096	63.8	.665	5:5
149 and 150.....	5.78	2.64	117.5	1.615	31.8	.434	5:2.2
151 and 152.....	2.897	1.328	172.0	1.402	50.0	.407	5:2.5
155 and 156.....	.732	.344	114.5	1.138	50.0	.517	5:3.7
157 and 158.....	4.44	2.0	103.4	1.098	67.6	.718	5:5.4
159 and 160.....	.666	6.0	75.0	.785	76.5	.803	5:8.5
161 and 162.....	.666	10.0	102.0	1.381	73.6	1.00	5:6
163.....	20.47	12.7	104.4	1.068	69.4	.711	5:5.5

## ALFALFA, THIRD CROP

147.....	.014	.016	48.0	.854	21.22	.378	5:3.6
149 and 150.....	5.78	2.64	110.0	.974	31.6	.291	5:2.4
151 and 152.....	2.897	1.328	110.2	.922	32.2	.27	5:1.9
155 and 152.....	.732	.344	78.75	.955	29.62	.32	5:2.8
157 and 158.....	4.44	2.0	85.0	.902	40.7	.461	5:4
159 and 160.....	.666	6.0	40.8	.922	39.8	.783	5:7
161 and 162.....	.666	10.0	51.6	.87	54.7	.922	5:9.2
163.....	20.47	12.7	96.5	.99	49.0	.503	5:4.2

EFFECT OF MAGNESITE AND DOLOMITE UPON WHEAT AND SOYBEANS  
(SERIES I AND J)

Series I had wheat grown in the pots and then turned under, and wheat was then replanted in the same pots; while series J had cowpeas grown and turned under and then soybeans planted, except in pots 182 and 183, from which the cowpeas were removed before the soybeans were planted. The cowpea hay grown in pots 182 and 183 contained 0.4 per cent of calcium and 0.179 per cent of magnesium, in a ratio of 5 to 5.7, and removed from each pot 32 mgm. of calcium and 14.32 mgm. of magnesium. In 10 seeds planted there was 0.58 mgm. of calcium and 1.59 mgm. of magnesium. The above pots contained extracted sand.

Figure 1 of Plate LXXXV shows the effect of succeeding crops when grown upon extracted sand. The pot at the left marked "sand only" has had no other crop preceding it, while in the middle pot cowpeas were grown and removed, taking out some of the most readily available calcium and magnesium. From the pot at the right three crops of alfalfa were removed, taking out 164.5 mgm. of calcium and 90.72 mgm. of magnesium.

Dolomite has no detrimental effect upon the crops used throughout these experiments. However, the addition of larger quantities of magnesite—for example, 35 per cent—caused considerable yellowing of the leaves, and the plants were able to mature but few seeds. Plate LXXXV, figure 2, shows that the plants growing in dolomite have quite a number of bean pods, while in the magnesite pot none are visible and the uppermost leaves are sickly. This yellowing of the uppermost leaves while the lower ones remain green differs from true translocation and accompanies high magnesium applications. The yellow leaves have a higher magnesium content than do the healthy ones, as sickly leaves from the plants taken from pot 185 show 0.955 per cent of calcium and 1.11 per cent of magnesium, while the healthy leaves from the same plants showed 0.896 per cent of calcium and 0.88 per cent of magnesium, respectively.

Schulze and Godet (31) found more calcium in the husk and more magnesium in the seed of lupine, pine, pumpkin, castor bean, sunflower, and various nuts.

Plate LXXXVI, figure 1, shows the comparative growths of soybeans in brown silt loam and dolomite. Evidently the brown silt loam would have been improved by applications of some limestone or dolomite.

The differences of yields of duplicates in Table XI are due chiefly to the differences in the duration of growth. In the wheat, series I, pots 173, 175, 177, and 179 were harvested 65 days after planting, while their duplicates were harvested 12 days earlier. In the soybean, series J, pots 181, 184, 186, and 188 were harvested 53 days after planting, while their duplicates were permitted to mature, standing until 80 days after planting.

TABLE XI.—Yields of wheat and soybeans (in grams per pot on the water-free basis) in dolomite, magnesite, and sand—series I and J

Treatment.	Molecular ratio of calcium to magnesium.	Wheat, series I.			Soybeans, series J.		
		Pot No.	Tops.	Roots.	Pot No.	Tops.	Roots.
None.....	{.....	173	0.9	0.4	181	1.0	0.5
	{.....	174	.3	.4	182	1.2	.....
Percentage of magnesium in magnesite plus calcium carbonate:							
2.....	{ 5:4	175	4.8	2.9	183	5.6	.....
	{ 5:4	176	1.6	1.1	184	4.7	.9
10.....	{ 5:125	177	5.3	1.7	185	4.0	.....
	{ 5:125	178	1.8	1.0	186	3.2	.8
Percentage of magnesium in dolomite C <sub>3</sub> :							
12.....	{ 5:5.2	170	5.3	3.2	187	6.3	.....
	{ 5:5.2	180	1.9	.9	188	3.4	.7

Table XII shows the analyses of wheat grown in series I.

TABLE XII.—Analysis of straw of wheat grown in dolomite, magnesite, and sand—series I

Pot No.	Substance added.		Composition of plants.				
	Calcium.	Magnesium.	Calcium.		Magnesium.		Molecular ratio of calcium to magnesium.
	Per cent.	Per cent.	Mgm.	Per cent.	Mgm.	Per cent.	
174.....	0.014	0.016	1.32	0.44	0.38	0.127	5: 2.4
173.....	.014	.016	1.21	.135	1.26	.14	5: 7.1
170.....	4.44	2.0	10.88	.68	13.04	.815	5: 9.9
175.....	4.44	2.0	21.36	.445	36.78	.783	5:14.6
178.....	.666	10.0	8.87	.403	18.03	1.002	5:16.9
177.....	.666	10.0	16.16	.305	40.54	.765	5:21
180.....	20.47	12.7	10.26	.54	11.38	.599	5: 9.2
179.....	20.47	12.7	24.11	.455	22.15	.418	5: 7.6

Comparisons of the contents of plants at different stages of growth are reported in Table XIX.

#### THE EFFECT OF MAXIMUM QUANTITY OF CALCIUM AND MAGNESIUM UPON WHEAT AND SOYBEANS IN SAND (SERIES K)

Analysis of sand treated by different methods shows the hot-extracted sand to contain only slightly less calcium but considerably less magnesium than the cold-extracted sand.

Table XIX shows the analysis of wheat and soybeans grown in such sands. It can be seen that the soybeans contained only slightly more of these two elements than was in the seed, but it must be remembered that scarcely any growth occurred. However, the wheat, pots 193 to 196, contained from 12 to 22 times as much calcium and 4 times as much

magnesium as was added in the seed. Now, in pots 199 to 202, where a small amount of easily available calcium had been applied, the percentage in the plants was materially increased.

Attempts were made to grow wheat and cowpeas in paraffin, so that they would have no access to calcium and magnesium. However, this permitted but little growth, and analyses of the total plants thus grown showed their calcium and magnesium contents to be equivalent to the amount present in the seed.

TABLE XIII.—*Analysis of wheat and soybeans grown in extracted sand—series K*

SOYBEAN PLANT

Pot No.	Treatment of sand.	Composition of plants.					Substance added in seed.	
		Calcium.		Magnesium.		Molecular ratio of calcium to magnesium.	Cal- cium.	Magne- sium.
		Mgm.	Per ct.	Mgm.	Per ct.		Mgm.	Mgm.
189 and 190 <sup>a</sup>	Extracted with hydrochloric acid in the cold.	2. 15	0. 293	2. 77	0. 376	5: 10. 6	1. 16	2. 32
191 and 192	Extracted with hydrochloric acid on steam bath.	1. 5	. 335	1. 65	. 369	5: 9. 2	1. 16	2. 32

WHEAT PLANT

193 and 194	Extracted with hydrochloric acid in the cold.	. 67	. 22	. 78	. 256	5: 9. 7	. 03	. 18
195 and 196	Extracted with hydrochloric acid on steam bath.	. 36	. 168	. 72	. 337	5: 16. 7	. 03	. 18
197 and 198	0.2 gm. sodium bicarbonate (NaHCO <sub>3</sub> ).	. 80	. 155	. 74	. 144	5: 8	. 03	. 18
199 and 200	0.2 gm. sodium bicarbonate (NaHCO <sub>3</sub> ) 0.05 gm. calcium nitrate (Ca(NO <sub>3</sub> ) <sub>2</sub> ).	1. 14	. 3	. 35	. 093	5: 2. 5	. 03	. 18
201 and 202	0.2 gm. sodium bicarbonate (NaHCO <sub>3</sub> ) 0.05 gm. calcium nitrate (Ca(NO <sub>3</sub> ) <sub>2</sub> ) 0.0317 gm. magnesium sulphate (MgSO <sub>4</sub> )	. 34	. 247	. 48	. 35	5: 11. 8	. 03	. 18

<sup>a</sup> The containers in this series were tall Jena beakers holding 1,350 gm. of sand.

From Table XIII it can be seen that the plants contained more calcium and magnesium than was added in the seed, thus showing their power to obtain these two elements from sand that had been previously extracted with acid.

## EFFECT OF MAGNESIUM AND CALCIUM IN SULPHATES, CHLORIDS, AND CARBONATES UPON WHEAT AND SOYBEANS IN SAND (SERIES L AND M)

When calcium and magnesium were applied in sulphates, chlorids, and carbonates the smaller applications gave the highest yields. As recorded in Table XIV, 0.1 per cent of magnesium in the carbonate inhibited germination and permitted no growth, whereas this quantity in the sulphates and chlorids gave considerable growth; however, the chlorids were more detrimental than the sulphates, while at lower concentrations, such as 0.01 and 0.001 per cent of magnesium, the carbonates gave the best growth, the chlorids being the most detrimental. In the case of soybeans all the chlorid treatments permitted practically no seed formation, while treatment with smaller quantities of carbonates gave considerable seed. The root formation was relatively the same as the top growth, the detrimental effect accompanied short thick roots which appeared brownish or reddish brown. Plate LXXXVII shows this comparative root growth. Plate LXXXVIII, figure 1, shows the comparisons of wheat when grown in extracted sand and in dolomite. Figure 2 shows the retarded growth of wheat due to the chlorids of magnesium.

TABLE XIV.—Yields of wheat and soybeans (in grams per pot on the water-free basis) in the sulphates, chlorids, and carbonates of magnesium and calcium—series L and M

Treatment.	Molecular ratio of calcium to magnesium.	Wheat, series L.			Soybeans, series M.		
		Pot No.	Tops.	Roots.	Pot No.	Tops.	Roots.
None.....		219	5.8	3.0	237	1.3	0.3
None.....		220	2.1	1.0	238	2.0	.....
Percentage of magnesium in magnesium sulphate plus calcium sulphate:							
0.1.....	{ 5:4	203	2.4	1.4	221	3.2	.....
	{ 5:4	204	3.3	1.1	222	2.6	.6
.01.....	{ 5:4	205	3.8	1.5	223	5.5	.....
	{ 5:4	206	4.4	1.3	224	4.9	1.0
.001.....	{ 5:4	207	5.1	1.7	225	3.6	.7
	{ 5:4	208	3.8	1.6	226	4.0	.....
Percentage of magnesium in magnesium chlorid plus calcium chlorid:							
0.1.....	{ 5:4	209	1.6	.7	227	2.4	.....
	{ 5:4	210	1.5	.6	228	2.1	.6
.01.....	{ 5:4	211	3.1	1.3	229	2.6	.0
	{ 5:4	212	3.5	1.6	230	2.7	.6
.001.....	{ 5:4	213	5.2	2.5	231	3.6	1.0
	{ 5:4	214	8.8	4.7	232	3.5	.....
Percentage of magnesium in magnesium carbonate plus calcium carbonate:							
0.01.....	{ 5:4	215	5.8	4.0	233	2.9	.7
	{ 5:4	216	3.3	1.5	234	7.8	.....
.001.....	{ 5:4	217	6.8	7.8	235	6.3	1.2
	{ 5:4	218	4.2	2.6	236	7.6	.....



In Table XIV pots 205, 208, 210, 212, 213, 216, 218, and 220 were harvested 53 days after planting. Their duplicates were permitted to grow 12 days longer. Pots 221, 223, 226, 227, 229, 232, 235, and 237 were harvested at maturity, 80 days from the time of planting. Duplicates were grown only 53 days. The analyses are given in Tables XV and XVI.

TABLE XV.—*Analysis of straw of wheat grown with sulphates, chlorids, and carbonates of calcium and magnesium—series L*

Pot No.	Substance added.		Composition of plants.				Molecular ratio of calcium to magnesium.
	Calcium.	Magnesium.	Calcium.		Magnesium.		
			Mgm.	Per cent.	Mgm.	Per cent.	
203.....	0.222	0.1	18.48	0.77	15.45	0.644	5: 6.9
205.....	.022	.01	26.6	.70	38.86	.97	5:11.5
208.....	.002	.001	15.2	.40	5.73	.151	5: 3.1
SULPHATES							
210.....	.222	.1	22.5	1.5	11.77	.785	5: 4.3
212.....	.022	.01	12.67	.362	9.66	.276	5: 6.8
213.....	.002	.001	22.36	.43	9.05	.174	5: 3.3
CHLORIDS							
216.....	.022	.01	9.24	.28	20.33	.616	5:18.3
218.....	.002	.001	17.28	.432	15.68	.392	5: 7.5
220.....	.....	.....	5.31	.253	2.58	.123	5: 4
CARBONATES							

The plants used in the experiments in Table XV were harvested when 53 days old.

TABLE XVI.—*Analysis of soybean hay grown with sulphates, chlorids, and carbonates of calcium and magnesium—series M*

Pot No.	Substance added.		Composition of plants.				Molecular ratio of calcium to magnesium.
	Calcium.	Magnesium.	Calcium.		Magnesium.		
			Mgm.	Per cent.	Mgm.	Per cent.	
222.....	0.222	0.1	30.94	1.19	31.46	1.21	5:8.4
224.....	.022	.01	41.89	.885	31.45	.642	5:6.2
225.....	.002	.001	21.24	.59	12.24	.34	5:4.8
SULPHATES							
228.....	.222	.1	15.39	.733	13.96	.665	5:7.5
230.....	.022	.01	25.11	.93	19.54	.724	5:6.4
231.....	.002	.001	13.89	.386	10.26	.285	5:6.1
CHLORIDS							
233.....	.022	.01	27.26	.94	23.92	.825	5:7.3
235.....	.002	.001	72.13	1.145	38.36	.609	5:4.4
237.....	.....	.....	7.15	.55	4.04	.311	5:4.7
CARBONATES							

## EFFECT OF MAGNESIUM AND CALCIUM IN CALCAREOUS SOIL, DOLOMITE, AND MAGNESITE, AFTER ALFALFA, UPON SOYBEANS (SERIES N)

The soybeans in series N were grown after three crops of alfalfa had been removed and the roots turned under. Pots 239 and 240 showed but a small amount of growth. Pots 251, 252, and 253 showed considerable organic growth, but the plants were sickly and did not yield much seed. The yields are reported in Table XVII. Analyses of the plants, Table XVIII, show treatments with the largest quantities of magnesium in magnesite, giving the plants with the greatest magnesium content and containing as much as 29.92 pounds of magnesium per ton. Also proportionately the highest amount of calcium and magnesium were found in these pots. The check pots, 239 and 240, showed the lowest percentage of calcium and magnesium in the plants grown.

TABLE XVII.—Yields of soybeans (in grams per pot on the water-free basis) grown after alfalfa in soil, magnesite, and sand—series N.

Treatment.	Molecular ratio of calcium to magnesium.	Soybeans, series N.			
		Pot No.	Tops.	Roots.	Seeds.
None		239	1.3		
		240	1.6		
Percentage of magnesium in calcareous soil:					
2.64	5:3.8	241	5.6	0.6	
	5:3.8	242	7.3		2.51
1.328	5:3.8	243	7.2	1.2	
	5:3.8	244	6.2		2.2
.672	5:3.8	245	8.5		4.59
	5:3.8	246	6.1	1.2	
.344	5:3.9	247	12.1		6.95
	5:3.9	248	6.5	1.0	
Percentage of magnesium in magnesite plus calcium carbonate:					
0.2	5:4	249	6.6		1.84
	5:4	250	6.6	2.0	
10	5:125	252	3.8	.7	
	5:125	253	8.5		
Percentage of magnesium in magnesite:					
0.6	5:75	251	4.0	.7	
Percentage of magnesium in dolomite C <sub>3</sub> :					
12.7	5:5.2	254	3.8	.7	

Pots 240, 242, 244, 245, 247, 249, and 253 were harvested at maturity, or 80 days after planting.

The plants used in the experiments in Table XVIII were 53 days old.

Table XIX shows the differences in composition of wheat grown under the same treatment but harvested at different periods of growth. The first plants were harvested 53 days after being planted. It was the original plan to allow the duplicates to mature, but owing to attacks of mildew they were harvested 12 days later.

TABLE XVIII.—Analysis of soybeans grown after alfalfa in soil, magnesite, and sand—series N

Pot No.	Substance added.		Composition of plants.				Molecular ratio of calcium to magnesium.
	Calcium.	Magnesium.	Calcium.		Magnesium.		
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	<i>Mgm.</i>	<i>Per cent.</i>	
239.....	0.014	0.016	7.02	0.54	4.6	0.354	5: 5.4
241.....	5.78	2.64	109.76	1.06	41.5	.741	5: 3.1
243.....	2.897	1.328	131.0	1.82	52.7	.732	5: 3.3
246.....	1.455	.672	90.28	1.48	47.88	.785	5: 4.4
248.....	.732	.344	76.62	1.225	48.42	.745	5: 5.1
250.....	4.44	2.0	82.83	1.225	76.54	1.19	5: 7.9
251.....	.666	6.0	29.04	.726	30.40	.985	5:11.3
252.....	.666	10.0	32.83	.864	50.85	1.496	5:14.4
254.....	20.47	12.7	46.96	1.236	31.73	.835	5: 5.6

The percentages of calcium and magnesium were greater in the plants harvested in the earlier stages of growth. In the wheat the proportion of magnesium to calcium was somewhat greater in the later stages of growth. Still it must be remembered that the plants were by no means thoroughly matured. This was not the case with the soybeans, as is shown by Table XX. Soybean plants at maturity, or 80 days after planting, showed higher percentages of calcium and magnesium than at the end of 53 days of growth, except the checks in sand and those having had extremely small applications.

TABLE XIX.—Composition of wheat at different stages of growth

Treatment.	Wheat 53 days old.				Wheat 65 days old.		
	Pot No.	Calcium.	Magnesium.	Molecular ratio of calcium to magnesium.	Calcium.	Magnesium.	Molecular ratio of calcium to magnesium.
None.....	173	0.44	0.127	5: 2.4	0.135	0.14	5: 7.1
Percentage of magnesium in dolomite C <sub>3</sub> : 12.7.....	179	.54	.599	5: 9.2	.455	.418	5: 7.6
Percentage of magnesium in magnesite: 10.....	177	.403	1.002	5:16.9	.305	.765	5:21
2.....	175	.68	.815	5: 9.9	.445	.783	5:14.6
Percentage of magnesium in magnesium sulphate: 0.1.....	203	.77	.644	5: 6.9	.61	.54	5: 7.3
0.01.....	205	.70	.97	5:11.5	.40	.646	5:13.4
0.001.....	207	.40	.151	5: 3.1	.255	.158	5: 5.1
Percentage of magnesium in magnesium chlorid: 0.1.....	209	1.5	.785	5: 4.3	.984	.604	5: 5
0.01.....	211	.362	.276	5: 6.8	.30	.31	5: 8.6
0.001.....	213	.43	.174	5: 3.3	.245	.163	5: 5.5
Percentage of magnesium in magnesium carbonate: 0.01.....	215	.28	.616	5:18.3	.405	.587	5:12
0.001.....	217	.432	.392	5: 7.5	.455	.316	5: 5.8
None.....	219	.253	.123	5: 4	.15	.16	5: 8.8

Seissl (32) experimented with a large number of plants in various stages of growth and found a slight fluctuation in the ratio of calcium to magnesium in the ash analyzed in the different years. In nearly every instance there was a progressive increase in the ratio of the lime to the magnesia content towards autumn. In only two cases was the lime content greater than that of the magnesia.

TABLE XX.—Composition of soybeans at different periods of growth

Treatment.	Pot No.	Soybeans 53 days old.			Soybeans 80 days old.		
		Calcium.	Mag- nesium.	Molecular ratio of calcium to mag- nesium.	Calcium.	Mag- nesium.	Molecular ratio of calcium to mag- nesium.
None.....	181	<i>Per cent.</i> 0.344	<i>Per cent.</i> 0.449	5:10.8	<i>Per cent.</i> 0.29	<i>Per cent.</i> 0.28	5: 8
Percentage of magnesium in dolomite C <sub>3</sub> :							
12.7.....	187	1.546	.964	5: 5.2	2.07	1.3	5: 5.2
Percentage of magnesium in magnesite:							
10.....	185	.96	1.032	5: 9	.75	1.138	5:12.6
2.....	183	1.357	.838	5: 5.2	1.15	.815	5: 5.9
Percentage of magnesium in magnesium sul- phate:							
0.1.....	221	1.19	1.21	5: 8.4	1.71	2.28	5:11.1
.01.....	223	.855	.642	5: 6.2	1.215	1.31	5: 9
.001.....	225	.59	.34	5: 4.8	.6	.356	5: 4.8
Percentage of magnesium in magnesium chlorid:							
0.1.....	227	.733	.665	5: 7.5	2.55	1.33	5: 4.3
.01.....	229	.93	.724	5: 6.4	.65	.495	5: 6.3
.001.....	231	.386	.285	5: 6.1	.27	.255	5: 7.9
Percentage of magnesium in magnesium car- bonate:							
0.01.....	233	.94	.825	5: 7.3	1.13	1.19	5: 8.7
.001.....	235	1.145	.609	5: 4.4	1.57	.399	5: 2.1
None.....	237	.55	.311	5: 4.7	.36	.184	5: 4.2
Percentage of magnesium in calcareous soil:							
2.64.....	241	1.06	.741	5: 3.1	3.0	.997	5: 2.77
1.32.....	243	1.82	.737	5: 3.3	3.65	1.26	5: 2.87
.672.....	245	1.48	.785	5: 4.4	2.7	1.016	5: 3.1
.344.....	247	1.225	.745	5: 5.1	2.11	.79	5: 3.1
None.....	239	.54	.354	5: 5.4	.17	.118	5: 9.2
Percentage of magnesium in magnesite:							
2.....	249	1.255	1.10	5: 7.9	1.55	1.55	5: 8.3
10.....	252	.864	1.496	5:14.4	1.145	2.166	5:15.75

TABLE XXI.—*Tolerance of crops for calcium and magnesium*

Treatment.	Molecular ratio of calcium to magnesium.	Wheat.			Alfalfa.		
		Calcium.	Magnesium.	Molecular ratio of calcium to magnesium.	Calcium	Magnesium.	Molecular ratio of calcium to magnesium.
None.....	5:9.5	0.187	0.108	5: 5.1	0.347	0.164	5:3.9
Percentage of magnesium in dolomite C1:							
0.2.....	5:4.8	.298	.271	5: 7.6	2.565	.431	5:1.4
0.6.....	5:4.8	.386	.421	5: 9.1	2.622	.649	5:2
Percentage of magnesium in brown-gray silt loam:							
0.352.....	5:9.6	.296	.256	5: 7.2	1.595	.398	5:2.2
Percentage of magnesium in dolomite C3:							
12.7.....	5:5.2	.574	.730	5:12.6	1.068	.711	5:5.5
Percentage of magnesium in magnesite:							
10.....	5:125	.375	.935	5:21	1.381	1.00	5:6

The yields in the pots in Table XXI were practically the same on the different treatments. This shows the alfalfa to contain more calcium and magnesium than the wheat. Some of the other treatments show higher percentages of calcium and magnesium, but the yields are not comparable. It might be interesting to note that soybean hay at maturity contained per ton as much as 73 pounds of calcium and 25.2 pounds of magnesium when grown in a mixture of equal parts of sand and calcareous soil, but when grown in a mixture containing 40 per cent of magnesite there were 22.9 pounds of calcium and 43.3 pounds of magnesium per ton.

DISCUSSION

The experiments reported here extend over a period of three years (1912 to 1915) and include approximately 300 pot cultures and upwards of 300 duplicate determinations of calcium and magnesium.

Difficulty was experienced in finding a medium that was free from calcium and magnesium, and which would still approach soil conditions. Attempts were made to grow plants in aluminum turnings but without success, probably due to the formation of some aluminum salts when the plant foods were added. It is well known that aluminum salts disturb the physiological functioning of plant organs.

Wheat and cowpeas grown in granular paraffin without the addition of calcium and magnesium showed in the total plant only an amount equal to that furnished by the seed.

The difference in the medium in which the plants were grown caused different effects upon the plants. Brown silt loam was a better medium than sand when treated with chemically pure magnesium carbonate, even though it already contained 25 times as much calcium and magnesium as did the sand. Still sand would have an ameliorating effect when compared with solution cultures. Jensen (10) found that in quartz sand a much higher concentration of salts was required to cause death than in water cultures.

As previously shown under literature studies it is quite generally believed that plants have to some extent a selective absorption. The results here seem to indicate such a condition, for the dolomites used tend to go into solution in a molecular ratio, but the plants failed to take them up in this ratio. The tendency of the plants under these conditions was to take up relatively larger molecular proportions of magnesium than of calcium. Analysis of the plants show that they do not necessarily take up calcium and magnesium in the same ratio as applied, as, for example, in dolomite C<sub>3</sub> the ratio of calcium to magnesium is 5:5.2, while the plants may and do take it up in a ratio of 5:7 or 5:3.95.

In the case of the addition of 25 per cent of magnesite the ratio of calcium to magnesium was 5:125, while in some of the plants grown in such treatment the ratio varied from 5:15 to 5:21. Wheat grown in soil treated with 6 per cent of dolomite showed in the tops a ratio of 5:9.1 and in the roots a ratio of 5:4.35, or for the whole plant a ratio of 5:6.3, while in dolomite C<sub>1</sub> it was 5:4.8. Alfalfa grown in the same treatment showed for the entire plant a ratio of 5:4.2, but when grown in soil treated with dolomite C<sub>3</sub> the ratio for the total alfalfa plant was 5:3.95, while in the dolomite the ratio of the calcium to the magnesium was 5:5.2.

The chlorids of calcium and magnesium were more detrimental to wheat and soybeans than were the sulphates at concentrations up to 0.1 per cent of magnesium. This amount of magnesium in the prepared carbonate entirely inhibited growth, whereas lower concentration gave better growth than either the sulphates or chlorids.

Wheat 65 days old showed smaller percentages of calcium and magnesium than did similarly treated wheat at 53 days of growth, but the total amount of these two elements in the plants increased with the duration of growth.

Soybeans at maturity, or 80 days after planting, showed for the hay higher calcium and magnesium contents than at 53 days of growth, except in the case of the checks and those treated with extremely small quantities. Some of the samples showed as much as 73 pounds of calcium and 25.2 pounds of magnesium per ton when grown in a mixture of one-half sand and one-half calcareous soil, but when grown in soil containing 35 per cent of magnesite there were 22.9 pounds of calcium

and 42.5 pounds of magnesium per ton; whereas the checks contained 5.8 pounds of calcium and 5.6 pounds of magnesium.

Whenever excessive amounts of magnesium were applied, there was a characteristic appearance of yellow leaves. The uppermost leaves became yellow and gradually died, while the lower leaves remained green. This condition is characteristic of magnesium sickness and just the reverse of the effects produced by translocation processes.

The general tendency is for the percentages of calcium and magnesium in the plants to increase with the increase in size of application. Likewise a high magnesium content in the plant tends to accompany plant sickness, as sickly and healthy leaves from the same soybean plant showed, respectively, 1.11 per cent of magnesium as against 0.88 per cent magnesium.

All varieties of the seed used contained more magnesium than calcium, while ordinarily the remainder of the plant contained more calcium than magnesium. This conforms with the data of Schulze and Godet, who report more calcium in the husk and more magnesium in the seed.

Nitrogen was applied to the legumes as well as to the cereals, so as to be sure that this was not the limiting factor.

In a number of instances the differences in the yields between duplicates were as great as between the different treatments. At several periods during the growth of the plants parasites caused injuries, sometimes great enough to necessitate harvesting the crop.

#### CONCLUSIONS

(1) Wheat, soybeans, alfalfa, and cowpeas grew normally either in 96 per cent of dolomite and 4 per cent sand, 100 per cent of magnesian limestone, or in sand containing 7 per cent of magnesite.

(2) Dolomite up to 40 per cent proved beneficial to plant growth. These results indicate that dolomite and magnesian limestone will not be detrimental as applied in agricultural practices.

(3) Applications of prepared magnesium carbonate up to 0.7 per cent caused no injury in brown silt loam, but 0.35 per cent prevented the growth of all plants tested in sand.

(4) The crop yields and the ratio of calcium to magnesium in the plants bear no direct relation to the ratio in the natural carbonates applied.

(5) Different ratios of calcium to magnesium within rather wide limits produced no marked differences in yields.

(6) Increasing the size of applications increased the calcium and magnesium content of plants.

(7) A tolerance of calcium and magnesium occurred in all varieties of plants grown. With approximately identical yields, wheat straw

grown in sand, brown silt loam, dolomite, and soil containing 35 per cent of magnesite showed calcium contents varying between 0.165 per cent and 0.547 per cent and magnesium contents varying between 0.132 per cent and 0.955 per cent.

(8) Acid extractions failed to remove all the calcium and magnesium from the sand. There remained after the various extractions from 768 to 852 mgm. of calcium and from 540 to 960 mgm. of magnesium per 6,000 gm. of sand.

(9) The plants possessed a decided ability to obtain calcium and magnesium from sand extracted with strong hydrochloric acid, as borne out by the following example: Three crops of alfalfa removed from acid extracted sand 164.43 mgm. more calcium and 90.4 mgm. more magnesium than was contained in seeds similar to those planted.

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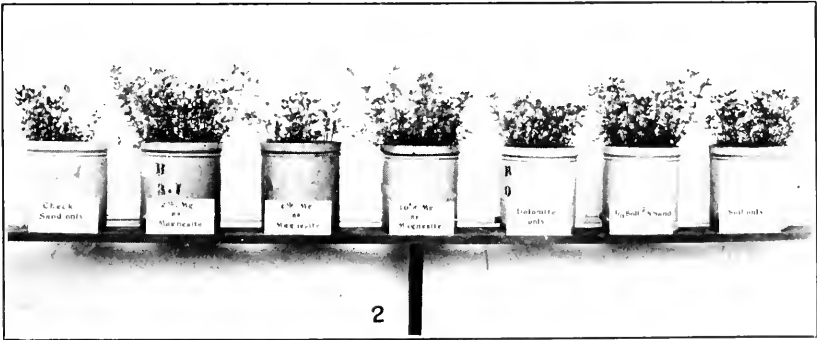
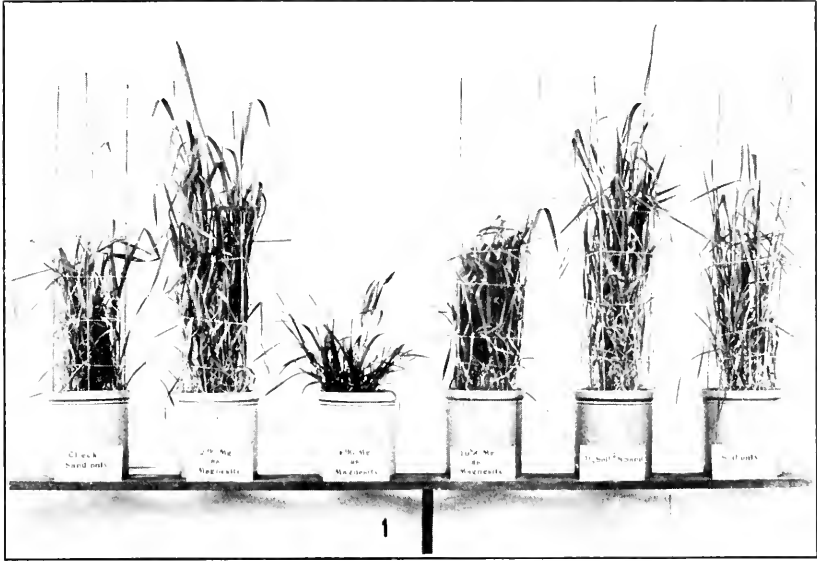
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PLATE LXXXIV

Fig. 1.—Growth of wheat in sand containing varying quantities of calcium and magnesium. The small growth of wheat in the pot marked "6% magnesium" is due to a detrimental physical effect.

Fig. 2.—Growth of alfalfa in sand containing varying amounts of calcium and magnesium.



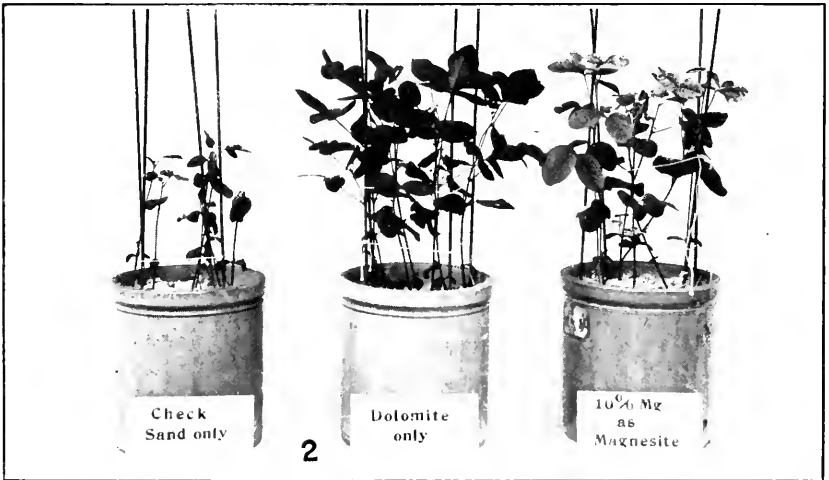
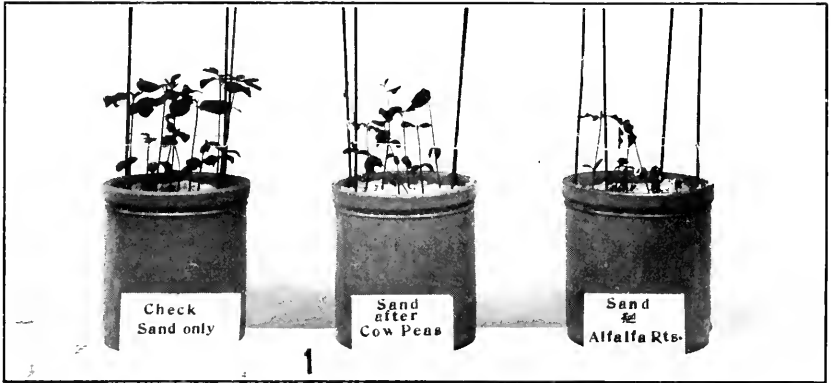


PLATE LXXXV

Fig. 1.—Growth of soybeans following a crop which had already absorbed most of the readily available calcium and magnesium.

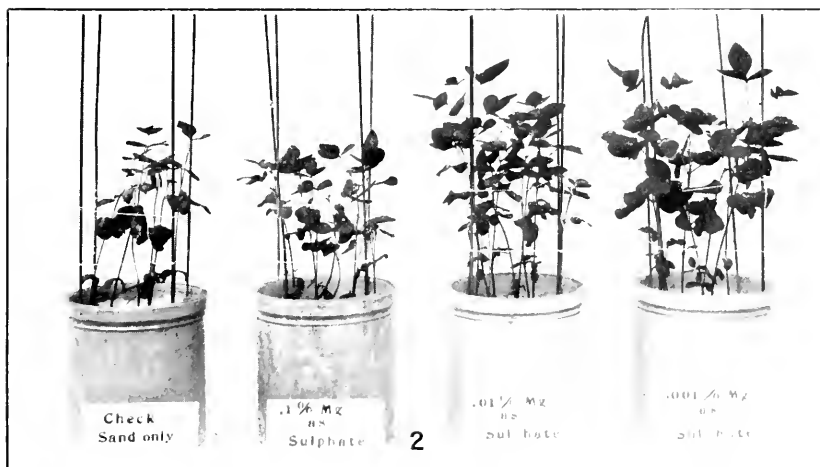
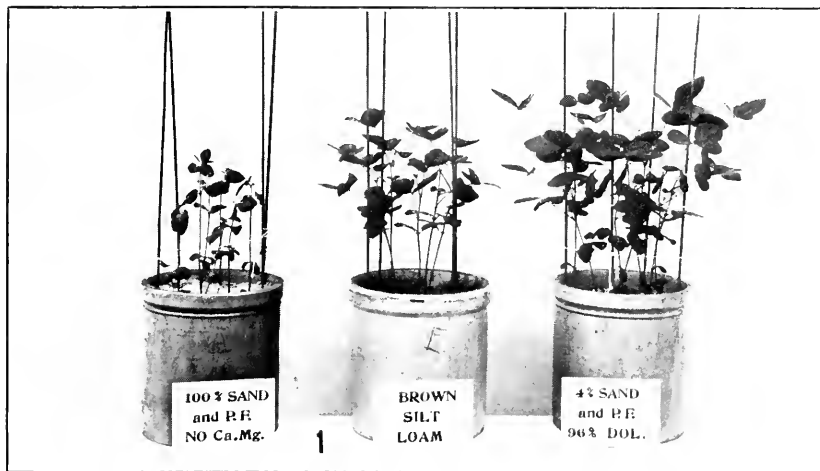
Fig. 2.—Growth of soybeans in soil treated with magnesium. Note the sickly appearance of the top leaves in the right-hand pot, which is characteristic of treatment with large quantities of magnesium.

PLATE LXXXVI

Fig. 1.—Comparative growth of soybeans in brown silt loam and dolomite, showing that the loam would have been improved by the addition of some limestone or dolomite.

Fig. 2.—Soybeans in sand treated with magnesium, showing that their growth increases inversely with the quantity of magnesium applied as sulphate.





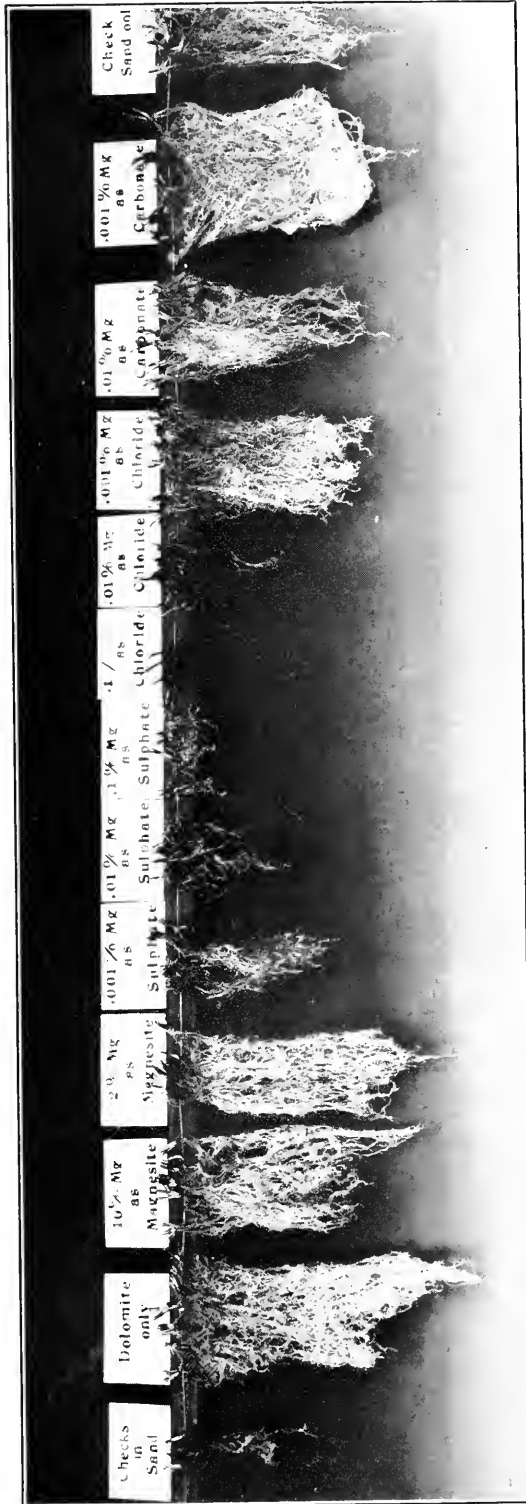


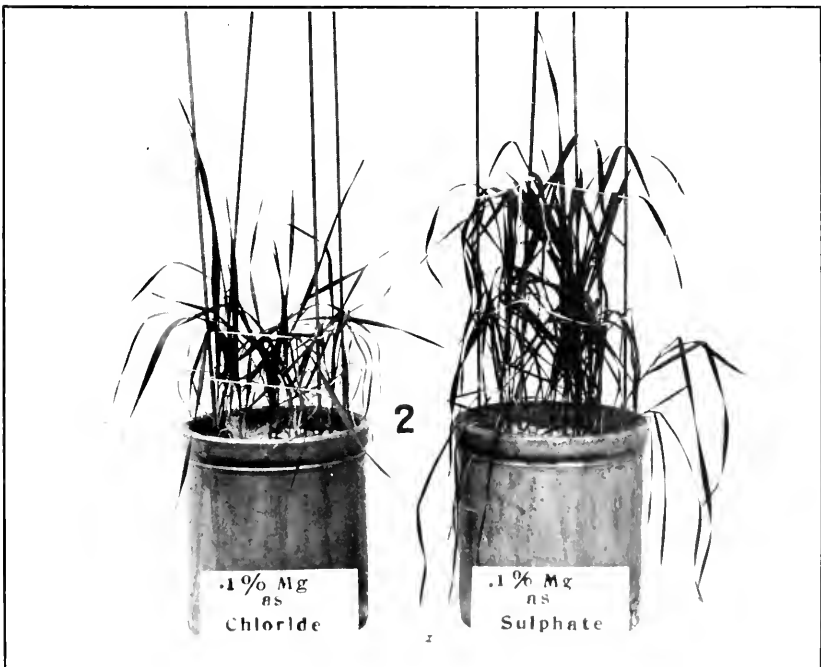
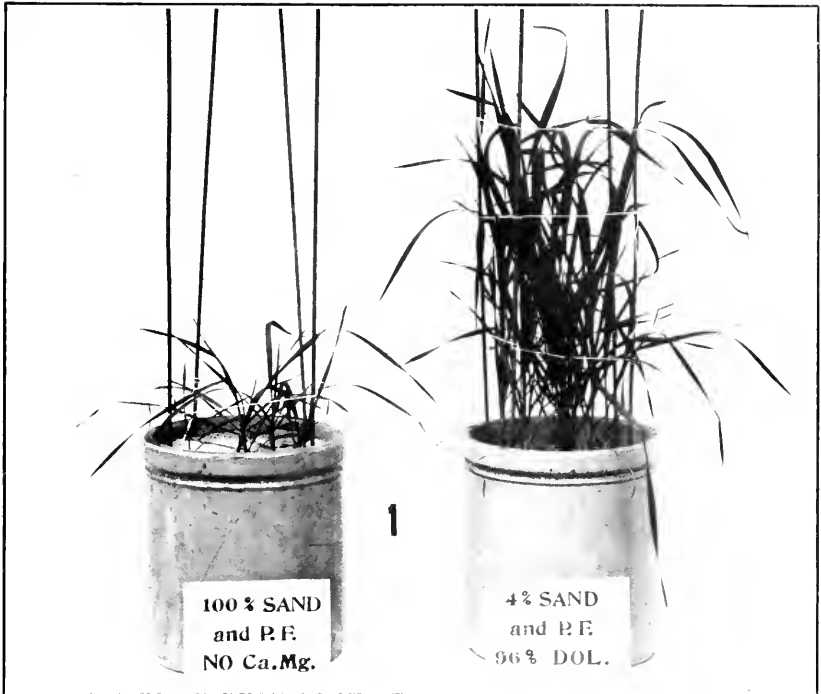
PLATE LXXXVII

Comparative root production of wheat grown in the chlorids, sulphates, and carbonates of magnesium and calcium. Root growth is inversely proportional to the amount of salt applied.

PLATE LXXXVIII

Fig. 1.—Comparative growth of wheat in sand and in dolomite. The dolomite contained 96 per cent of carbonates of calcium and magnesium and 4 per cent of insoluble silica residues.

Fig. 2.—Comparative growth of wheat in magnesium chlorid and magnesium sulphate.





# LARVAL CHARACTERS AND DISTRIBUTION OF TWO SPECIES OF DIATRAEA

By T. E. HOLLOWAY,<sup>1</sup>

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Bureau of Entomology

In May, 1911, Dyar<sup>2</sup> published an article differentiating the American species of *Diatraea*. The species *D. saccharalis*, commonly known as the sugar-cane moth borer and also as the larger corn stalk borer, which had been recorded in the literature as infesting corn and sugar cane throughout the Southern States, was divided into *D. saccharalis crambidoides* Fabricius and *D. zeacolella* Dyar. This paper came as a surprise to entomologists; and while in it Dyar scarcely mentions food plants it was assumed that *D. zeacolella* was supposed to infest corn (*Zea mays*) and not sugar cane (*Saccharum officinarum*), while *D. saccharalis crambidoides* was supposed to breed in sugar cane. The foundation for this belief, aside from the use of the term "zeacolella" for one of the forms, is evidently a statement made by Dyar before the Entomological Society of Washington in 1911,<sup>3</sup> which is noted in the Proceedings as follows:

Dr. Dyar spoke of the troublesome genus *Diatraea* and announced his success in separating as two distinct species the forms feeding on corn and sugar cane in the United States in characters of both the larvæ and the adults.

After the publication of Dyar's article, series of specimens were reared from corn and sugar cane at the laboratory of Sugar-Cane Insect Investigations at Audubon Park, New Orleans, La., and these were found by Mr. U. C. Loftin to interbreed. Specimens from both sugar cane and corn were determined by Dr. Dyar as *D. saccharalis crambidoides*.

Within the last two or three years, in the field investigations of sugar-cane insects, *D. saccharalis crambidoides* has been found to be limited to the southern half of Louisiana, including the southwestern corner of Mississippi around Woodville, to the southern half of Florida, and to the lower Rio Grande Valley in Texas. There was no doubt, however, about the correctness of the records of species of *Diatraea* from Virginia and the Carolinas, and the writer was at a loss to explain the divergence between his records and the statements in economic literature

<sup>1</sup> The writer gratefully acknowledges the assistance of Mr. August Busck and Rev. J. J. De Gryse, who very kindly criticized the drawings and descriptions and helped in many ways.

<sup>2</sup> Dyar, H. C. The American species of *Diatraea* Guilding (Lepid., Pyralide). *In* Ent. News, v. 22, no. 5, p. 199-206. 1911.

<sup>3</sup> Proc. Ent. Soc. Wash., v. 13, no. 2, p. 87. 1911.

that *D. saccharalis* occurs throughout the Southern States. Referring to Dyar's paper, he noticed that the range of *D. saccharalis crambidoides* is given as "Mexico, numerous localities, Gulf States, and lower Mississippi Valley," a range which roughly covers the limits which in numerous field trips have since been defined more exactly. Dyar records *D. zeacolella* only from points in North Carolina, South Carolina, and Virginia.

To compare larvæ from these sections with the more southern species, the writer obtained specimens from various members of the staff of the Bureau of Entomology from the following places: Columbia and Bennettsville, S. C., and Waynesboro, Ga. (E. R. Barber); Batesburg, S. C. (E. A. McGregor); Thomasville, Ga. (G. D. Smith). All these larvæ were from corn. A casual examination was sufficient to show that they differed from *D. saccharalis crambidoides*. The most apparent difference is that the larvæ (summer form) from the places mentioned above have a clean-cut black-and-white appearance, while larvæ of *D.*

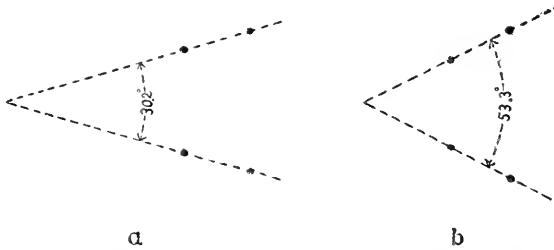


FIG. 1.—a, Average angle formed by imaginary lines through bases of setæ of *Diatraea saccharalis crambidoides*; b, average angle formed by imaginary lines through bases of setæ of *D. zeacolella*. Dots indicate bases of setæ.

*saccharalis crambidoides* (summer form), because of the lighter color of the tubercles, are of a more neutral color, which may be described as a kind of dirty white. That the strikingly marked larvæ were *D. zeacolella* was proved by an inflated specimen of the same species in the National Museum, which had been classified by Dr. Dyar as *D. zeacolella*. It was labeled, "On corn, Peacocks Store, N. C."

When the larvæ were shown to Mr. W. Dwight Pierce, of the Bureau of Entomology, he immediately observed a difference in the pattern of the dorsal tubercles (i and ii), the four tubercles of a segment of *D. zeacolella* roughly forming a trapezoid and those of *D. saccharalis crambidoides* forming a more rectangular figure. At the suggestion of Dr. W. D. Hunter, in Charge of Southern Field Crop Insect Investigations, and with the aid of Mr. August Busck, of the Bureau of Entomology, and Rev. J. J. De Gryse, at that time stationed at the Eastern Laboratory of Forest Insect Investigations, the writer proceeded to study the larvæ of the two species for further differences. While the pattern of the dorsal tubercles is valuable, their color fades in the winter, and it is then rather



difficult to determine the exact extent of the tubercles. The writer found that the positions of setæ i and ii correspond with the tubercles of the same numbers and that imaginary lines through the bases of the two setæ on each side form different angles in the two species. By the use of a camera lucida the relative positions of the bases of setæ i and ii on segments 3, 4, and 5 of 19 specimens of *D. saccharalis crambidoides* and of seven specimens of *D. zeacolcella* were determined, lines were drawn through the points representing the bases of the setæ, and the resulting angles were measured. It was found that the average angle for *D. saccharalis* was 30.2°, while for *D. zeacolcella* it was 53.3°. The size of these angles and the position of the lines through the bases of the setæ are graphically indicated in figure 1.

In different specimens the angles were found to vary from 41° to 69.5° in *D. zeacolcella*, and from 18° to 41.5° in *D. saccharalis crambidoides*. Angles of the minimum and maximum numbers of degrees are exceptional.

The differences in the larval characters are noted in the following comparison:

<i>Diatraea saccharalis crambidoides</i> Fabricius.	<i>Diatraea zeacolcella</i> Dyar.
Tubercles light brown or paler (summer form).	Tubercles dark brown, contrasting sharply with ground color of body (summer form).
Head brown, but may occasionally be yellow in winter form.	Head yellow.
Spiracles dark brown.	Spiracles black.
Abdominal tubercles i hardly twice as large as abdominal tubercles ii, more nearly equal.	Abdominal tubercles i about twice as large as abdominal tubercles ii.
Abdominal tubercles ii oval, and about twice as far apart as abdominal tubercles i.	Abdominal tubercles ii narrowed and about four times as far apart as abdominal tubercles i.
Two imaginary lines connecting bases of setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging 30.2°.	Two imaginary lines connecting bases of setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging 53.3°.

Descriptions of full-fed larvæ of both the summer and the winter forms of the two species are given below.

***Diatraea saccharalis crambidoides* Fabricius.**

**SUMMER FORM.**—Head rich brown, varying to black at mouth parts and to orange on dorsal aspect; slightly bilobed. Prothoracic plate pale brown, tinged with black ventrally, cephalic third of plate transparent. Body white. Segmentation distinct. Crochets biordinal. Tubercles light brown or paler, iv and v coalesced. Abdominal tubercles ii oval and about twice as far apart as tubercles i. Primary setæ yellow to brown. Imaginary lines connecting setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging 30.2°. No secondary setæ. Spiracles dark brown, elongate oval, distinct. Average length (10 specimens), 25.6 mm.

WINTER FORM.—Head yellow to rich brown, varying to black at mouth parts and to yellow on dorsal aspect; slightly bilobed. Prothoracic plate yellow. Body white. Segmentation distinct. Crochets biordinal. Tubercles white or pale yellow, and not easily distinguished from ground color of body, iv and v coalesced. Abdominal tubercles ii oval and about twice as far apart as tubercles i. Primary setæ yellow to brown. Imaginary lines connecting setæ i and ii, on each side, if prolonged, form angles averaging  $30.2^{\circ}$ . No secondary setæ. Spiracles dark brown, elongate oval, distinct and sharply contrasting with rest of body. Average length (10 specimens), 22.4 mm.

*Diatraea zeacolella* Dyar.

SUMMER FORM.—Head yellow, varying to black at mouth parts, slightly bilobed. Prothoracic plate yellow. Body white. Segmentation distinct. Crochets biordinal. Tubercles dark brown, contrasting sharply with ground color of body, iv and v coalesced. Abdominal tubercles ii narrowed and about four times as far apart as tubercles i. Primary setæ yellow to brown. Imaginary lines connecting setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging  $53.3^{\circ}$ . No secondary setæ. Spiracles black, elongate oval, distinct. Average length (3 specimens), 25.2 mm.

WINTER FORM.—Head yellow, varying to black at mouth parts, slightly bilobed. Prothoracic plate yellow. Body white. Segmentation distinct. Crochets biordinal. Tubercles white or pale yellow, and not easily distinguished from ground color of body, iv and v coalesced. Abdominal tubercles ii narrowed and about four times as far apart as tubercles i. Primary setæ yellow to brown. Imaginary lines connecting setæ i and ii of abdominal segments 3, 4, and 5, on each side, if prolonged, form angles averaging  $53.3^{\circ}$ . No secondary setæ. Spiracles black, elongate oval, very distinct and sharply contrasting with rest of body. Average length (4 specimens), 24.5 mm.

The writer has not seen all the instars of *D. zeacolella* Dyar. The instars of *D. saccharalis crambidoides* are similar, except the first, which is described below:

Head black, more horizontal than in following stages. Prothoracic plate dark brown. Body dirty white, widest at head and tapering caudally. Segmentation distinct. Prothoracic legs well developed. Elongate prolegs on protruding coxal lobes. Tubercles prominent. Primary setæ brown. No secondary setæ. Average length about 2 mm.

Not only do the larvæ of the two species vary in appearance, but their food plants and breeding habits differ to some extent. The food plants of *D. saccharalis crambidoides* include sugar cane, corn, and Johnson grass and other large grasses. Practically all of the larval period is spent within the stalks of the plants, except that the first instars feed about on the leaves. *D. zeacolella*, however, seems to have a preference for corn, even where sugar cane is present. Mr. U. C. Loftin found sugar cane at Thomasville, Ga., absolutely uninfested, but Mr. G. D. Smith had no trouble in obtaining larvæ from cornstalks. The writer has examined sugar cane at Waycross, Ga., without finding any larvæ. One larva only was found by Mr. Loftin at Chipley, Fla., in the sugar cane, and this is the only one recorded from sugar cane.

*D. zeacolella* goes far down in the taproots of corn, while *D. saccharalis crambidoides* does not have this habit. This was observed by Mr. E. R.

Barber and has been recorded (under *D. saccharalis*) by Howard<sup>1</sup> and Ainslie.<sup>2</sup>

The fact that *D. saccharalis crambidoides* has been found to be limited to such widely separated areas as southern Florida, southern Louisiana, and the southern tip of Texas will no doubt occasion some surprise. The explanation is that there is strong evidence tending to prove that the species was brought to this country in shipments of sugar cane from the Tropics and that it became established in the three sections in which sugar cane is an important crop. Both forms of larvæ of the two species are well shown in Plate LXXXIX.

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<sup>1</sup> Howard, L. O. The larger corn stalk-borer. *In* *Insect Life*, v. 4, no. 3/4, p. 95-103, fig. 2-4. 1891.

— The larger corn stalk-borer. U. S. Dept. Agr. Div. Ent. Circ. 16, 3 p., 3 fig. 1896.

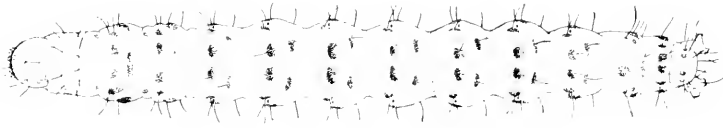
<sup>2</sup> Ainslie, G. G. The larger corn stalk-borer. U. S. Dept. Agr. Farmers' Bul. 634, 8 p., 4 fig. 1914.

PLATE LXXXIX

- Fig. 1.—*Diatraça saccharalis crambidoïdes*: Larva, summer form, dorsal view.  
Fig. 2.—*D. zecolella*: Larva, summer form, dorsal view.  
Fig. 3.—*D. saccharalis crambidoïdes*: Larva, summer form, side view.  
Fig. 4.—*D. zecolella*: Larva, summer form, side view.  
Fig. 5.—*D. saccharalis crambidoïdes*: Larva, winter form, dorsal view.  
Fig. 6.—*D. zecolella*: Larva, winter form, dorsal view.  
Drawn by Mr. Harry Bradford.



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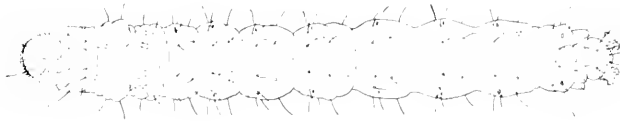
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# JOURNAL OF AGRICULTURAL RESEARCH

DEPARTMENT OF AGRICULTURE

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NO. 17

## THE DISEASE OF POTATOES KNOWN AS "LEAK"<sup>1</sup>

By LON A. HAWKINS,<sup>2</sup>

*Plant Physiologist, Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry*

### INTRODUCTION

The tuber-rot of potatoes (*Solanum tuberosum*) known as the "potato leak" is a disease of considerable importance in the delta region of the San Joaquin River, Cal. The rot is manifest in hot weather and appears soon after harvesting. As the potatoes in this region are sacked in the field and are practically all shipped immediately, the disease is therefore first evident in the car or warehouse. In extreme cases a whole shipment may be so badly damaged as to be worthless. If only a few "leakers" or "melters," as the rotten potatoes are called, are present it is usually necessary to sort the consignment. The cost of this sorting and the attendant shrinkage greatly increase the expense of production.

No exact data could be obtained as to the losses from this disease for any given season, but various estimates placed the damage for 1915 in the whole delta region, in which there were about 40,000 acres of potatoes, between \$50,000 and \$150,000. The general conditions and the methods of growing potatoes on these peat lands have been described by Orton (11),<sup>3</sup> Irish (8), and Shear (15). Orton and Shear have considered the diseases commonly found on potatoes in that region. In his paper Orton gives the results of a study of the potato leak, which is the only investigation of the disease heretofore reported. He was, however, prevented from completing the work on this disease to his satisfaction, and at his suggestion the writer took up the study.

In the study of the leak of potatoes described in the present paper it was planned to investigate further the causes of the disease, to study the organism or organisms causing it, their mode of entrance into the

<sup>1</sup> The work described in this article was carried out as a part of the potato-disease project of the Office of Cotton and Truck Disease Investigations.

<sup>2</sup> The writer's thanks are due Mr. W. V. Shear, of the Office of Horticultural and Pomological Investigations, for considerable assistance in the work at Stockton, Cal.

<sup>3</sup> Reference is made by number to "Literature cited," p. 639.

tuber, and, if possible, to obtain some data as to methods for its control. Part of the work was carried out at Stockton, Cal., and at various points in the delta potato fields near that city.

#### GENERAL APPEARANCE OF THE DISEASE

In taking up the study of the disease in the field, potatoes were examined at the sorting benches in the warehouses at Stockton, and various stages of the disease were observed. It was first apparent as a small brown discoloration around some wound, such as the wound made by the prong of a digging fork or by the breaking off of a "knob," which exposed the tissue of the inner part of the potato. The rot apparently did not affect tubers with unbroken skins. In the later stages of the disease the potatoes were brown over the entire surface, soft, and easily crushed. If sufficient pressure were applied to the tubers, a brownish watery liquid was exuded through breaks in the skin. Sacks containing potatoes in the advanced stages of this disease were frequently wet in patches where the rotten tubers had been crushed against the side. The interior of the rotten potato when broken was usually a dirty white, soon changing to a brown color around the edges. The center generally remained white for some time (Pl. XC).

#### ORGANISM CAUSING LEAK

##### RHIZOPUS NIGRICANS

Orton proved that the disease was caused by a fungus and concluded that the causal organism was *Rhizopus nigricans* Ehrenb. (10). He based his conclusions on the following premises: He observed a nonseptate mycelium in the rotted tubers and obtained *R. nigricans* in cultures made from these potatoes; he inoculated potatoes with this fungus and produced a rot similar in all appearances to leak.

That *R. nigricans* is able to rot Irish potatoes was also shown in unpublished studies by Mrs. Ethel Field Tillotson. In her experiments she used a strain of *R. nigricans* isolated from sweet potato (*Ipomoea batatas*). Her method of inoculation was to germinate the spores of the fungus in tubes of potato decoction and then pour the liquid, together with the germinated spores, into cavities in the potatoes. The inoculated tubers were placed in damp chambers, and in a few days the disease was evident.

With a strain of *R. nigricans* isolated from sweet potato by Mr. L. L. Harter the present writer was able to inoculate Irish potatoes successfully. The method developed by Mrs. Tillotson was followed in the earlier experiments. It was found unnecessary, however, to germinate the spores before inoculating the potatoes. Accordingly, in the later inoculations the tubers were inoculated directly from a culture of the fungus by inserting some of the spores and mycelium into rather deep wounds made in the tubers with a sterile knife. The inoculated potatoes were then placed in

a moist chamber and in from two to three days about 50 per cent showed evidences of the disease by brown coloration of the skin around the wound. In a week after inoculation the infected potatoes were usually entirely rotted. The skin was brown, and the interior of the potato was soft and watery. They were apparently typical leaky tubers. The fungus was readily reisolated from the rotten potatoes. From the investigations of Orton and the experiments of Mrs. Tillotson and of the present writer it is evident, then, that *R. nigricans* causes a rot of the Irish potato typical in appearance of the disease known as "leak." This work did not prove, however, that all cases of leak were due to *R. nigricans*, as it was very possible that other fungi acting in the same way might produce very similar results.

#### PYTHIUM DEBARYANUM

#### ISOLATION OF THE FUNGUS

Isolations of the fungus from potatoes were made by transferring portions of the partially rotted tubers obtained in the field to sterile tubes of slanted corn-meal agar and beef agar. In making these transfers the outer surface of the potato which had been washed in a 1 to 1,000 solution of mercuric chlorid was sliced away with a flamed knife and bits of the rotten portion of the potato farthest from the apparent point of infection were removed and placed in the culture tubes. In 24 hours a rather coarse hyalin mycelium was evident on the surface of the agar. After the cultures had grown for three days a microscopic examination of the fungus showed abundant fruiting bodies which much resembled the conidiospores of some species of *Pythium*. Occasionally structures were found which seemed to be oogonia and antheridia, though these were more frequently seen after a longer period. Transfers were made to the agar slants from 61 typical leaky tubers from a number of different fields. Of these transfers 49 proved to be cultures of this fungus, 5 of which were contaminated with bacteria. Six were cultures of bacteria only, and 6 were sterile. *R. nigricans* was not obtained in any of the cultures.

#### MORPHOLOGY OF THE FUNGUS

The fungus obtained from the leaky tubers was studied and found to be apparently a species of *Pythium*. The mycelium (fig. 1, *c*) of the fungus is rather coarse, irregularly branched, granular, usually nonseptate, though sometimes becoming septate when old. The conidia are borne either terminally or intercalary. They are usually nearly spherical when mature and are from 12 to 26 $\mu$  in diameter, averaging about 22 $\mu$ . They germinate immediately with one or more germ tubes when they are placed in water at ordinary room temperatures (fig. 1, *d*). The oogonia are spherical and borne like the conidia either terminally or intercalary.

They are from 15 to 25 $\mu$  in diameter, averaging about 22 $\mu$ . The antheridium (fig. 1, *b*) is borne either on the same filament as the oogonium or on an adjacent filament. If arising from the same filament it may be borne directly below the oogonium or some distance below. More than one antheridium was sometimes found attached to an oogonium. The oospores (fig. 1, *b*) are smooth, spherical, and thick-walled. They are from 14 to 19 $\mu$  in diameter, average 16 $\mu$ , and do not fill the oogonium. These measurements of the oogonia, oospores, and conidia all agree closely with those of *P. debaryanum*, as given by Butler. A culture of *P. debaryanum* used by Mr. C. P. Hartley in his studies on the damping-off of pine seedlings was obtained from the Office of Forest Pathology. This culture was a subculture of a strain which had been isolated from rotten

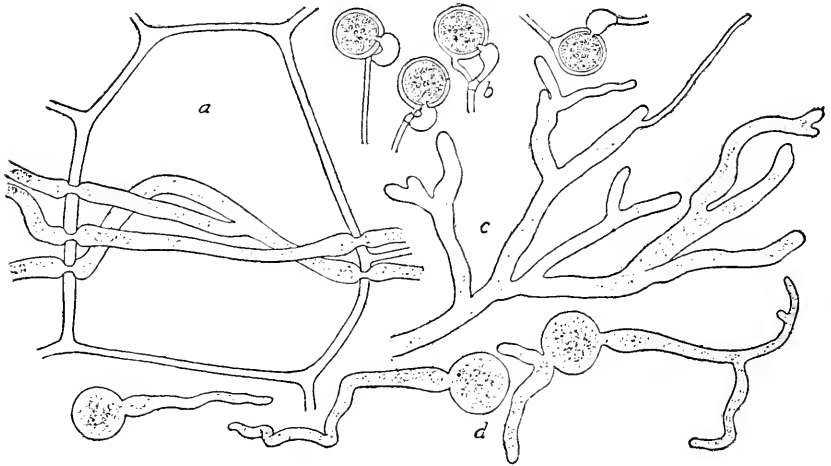


FIG. 1.—Microscopical appearance of *Pythium debaryanum* isolated from potatoes affected with potato leak: *a*, cell of a potato tuber showing fungus filaments therein; *b*, oogonia and antheridia; *c*, mycelium; *d*, germinating conidia.

potato by Edson (6) used by him in his studies on seedling diseases of sugar beets and then turned over to Hartley. This fungus agreed very closely with the *Pythium* sp. isolated from the leaky tubers in size of the conidia, oogonia, and oospores, habits of growth, and general appearance. Inoculated into potato tubers it produced a rot similar in all appearances to that produced by the fungus obtained from the leaky potatoes. It would seem, then, from the evidence above cited that the fungus isolated from the leaky potatoes in this study is the same as Hesse's *Pythium debaryanum* (7).

#### CULTURAL STUDIES

Cultures of the fungus were made on various kinds of media. The fungus grew well on beef, corn-meal, oatmeal, string-bean, Lima-bean, and potato agars, and Pfeffer's plant agar, potato plugs, and stems of *Melilotus alba*. Conidia and oogonia were formed when the fungus was

grown on string-bean and corn-meal agars, Pfeffer's plant agar, and the stems of *M. alba*. Neither conidia nor oogonia were found when the fungus was grown on the other kinds of media. The fungus produced both sexual and asexual reproductive bodies much more readily in Petri-dish cultures than in tubes. No sporangia or zoospores were seen in any of the cultures made in this study. It is of interest to note that Hesse (7), De Bary (2, 3), Sadebeck (13, 14), and Atkinson (1) are the only writers that to the author's knowledge record having observed the formation of zoospores by this fungus.

Cultures of the fungus were made from single conidia. To make these cultures some of the agar and mycelium from cultures which were producing conidia abundantly was ground up in sterile water. Corn-meal agar plates were poured in the usual way. The conidia germinated usually within an hour. The germinating spores were located by examining the inverted plates with a microscope. They were then marked and removed either to agar slants or to Petri dishes. The growth of these single-spore cultures was similar in all respects to that of the original 49 isolations of this fungus and to that of the strain of *P. debaryanum* obtained from the Office of Forest Pathology. They produced typical conidia, oogonia, and antheridia in abundance, and the mycelium showed the same characteristics as to branching and the granular structure of the protoplasm. Inoculations were made from these cultures into Burbank potatoes with positive results in 90 per cent of the cases. The fungus was reisolated from the rotted potatoes. The results obtained from these single-spore cultures indicate then that only the one fungus, *P. debaryanum*, was present in all the original 49 transfers.

The minimum, optimum, and maximum temperatures for growth and the temperatures at which growth was prevented were roughly determined for this fungus. For these experiments Petri-dish cultures on corn-meal agar were made from subcultures of five different isolations of the fungus and from the strain of *P. debaryanum* obtained from the Office of Forest Pathology. One Petri-dish culture for each constant temperature chamber was inoculated from subcultures from each isolation of the fungus. The growth of the cultures was measured each day for four days, after which the experiment was discontinued, as the culture media in some cases was entirely overgrown with mycelium.

The minimum temperature at which growth was noticeable in four days was between 5° and 8° C. No growth occurred at temperatures below 5°. The temperature at which growth is most rapid lies between 30° and 35°, and the maximum temperature at which growth can occur is between 35° and 40°. The fungus is killed at approximately 40°. Cultures from all five of the isolations from potatoes agreed as to these points, as also did the cultures from the strain of *P. debaryanum* obtained from the Office of Forest Pathology. The fungus was not killed at tem-

peratures below 5°, though growth was inhibited. The cultures from this chamber grew readily when placed in the incubator maintained at 30°. The experiments show that the range of temperature for growth is wide, about 30°, and that the optimum is high. Johnson (9) found the optimum temperature for growth of *P. debaryanum* to be 33°.

#### INOCULATION EXPERIMENTS

Inoculations were made into healthy California-grown Burbank potatoes from 30 of the 49 isolations of *P. debaryanum* obtained from diseased potatoes. Tubers were rotted and the fungus reisolated in all cases. Inoculations were also made with the bacterium which was sometimes obtained from the rotten tubers with no apparent effect. It seemed to be present as a saprophyte.

In the earlier inoculation experiments with *P. debaryanum*, the sterile tubers were inoculated with the fungus in wounds made with a flamed knife as in some of the experiments with *Rhizopus nigricans*. The inoculated tubers were then placed in moist chambers. Inasmuch as moist chambers, because of their limited volume of oxygen and their high humidity furnish rather abnormal conditions for the storing of potatoes, another method was developed in which the potatoes were maintained after inoculation under conditions which more nearly approached those found in storage. According to this method, the potatoes were disinfected as before and a small hole made in one side with a sterile knife. A ring, usually the ring of a Van Tieghem cell, was placed over the opening and cemented to the potato with petrolatum. A small quantity of sterile water was poured into the hole in the tuber and the inoculation made by placing some of the mycelium of the fungus in the water. A cover glass was then sealed on top of the cell with petrolatum. Various modifications of this method were tried to determine the size and depth of the wound necessary to insure a high percentage of successful inoculations. It was found that if the skin was removed from a small area of the potato which came within the ring when it was cemented in position and the inoculation made in a drop of sterile water on this wounded area, the results were as good as when deep wounds were made. Further experiments showed that it was sufficient to make a rather deep incision in the tuber with a sterile knife and introduce some mycelium to inoculate the potato successfully. The rots produced by such inoculations, however, became contaminated more frequently with bacteria than when the raw surface of the tuber was inclosed with a ring and cover glass. Numerous controls were prepared by cementing the ring to the unbroken surface of the tuber and placing therein some bits of mycelium in sterile water; also by pouring sterile water into wounds in the potatoes and sealing them as in the inoculation experiments. In none of these controls was there any infection.



In the inoculation experiments 210 sound potatoes of the Burbank variety were used, of which 177, or 84 per cent, were rotted.

Besides the experiments with California-grown potatoes, inoculation experiments were carried out with several eastern-grown varieties. These potatoes were kindly furnished by the Office of Horticulture and Pomological Investigations in most cases. The tubers were inoculated in deep wounds inclosed with a ring and cover glass, according to the method already described. Five different isolations of the fungus were used with each variety of potatoes. After inoculation they were placed in an incubator maintained at 30° C. and left there throughout the experiment. The results of this experiment are shown in Table I.

TABLE I.—Results of inoculating several varieties of eastern-grown potatoes with *Pythium debaryanum*, as shown by the number of potatoes of each variety rotted

Variety.	Number inoculated.	Number rotted.	Variety.	Number inoculated.	Number rotted.
Rose 4 (Florida) <sup>1</sup> . . . . .	17	13	Early Ohio . . . . .	11	10
Rose 4 . . . . .	9	0	Rural New York . . . . .	11	7
Early Rose . . . . .	12	5	Irish Cobbler . . . . .	12	5
Triumph . . . . .	9	5	Pearl . . . . .	12	3
Green Mountain . . . . .	10	7			

<sup>1</sup> Furnished by Mr. W. B. Clark, of the Office of Cotton and Truck Disease Investigations.

From the results shown in Table I it is evident that some of the varieties of eastern potatoes are about as susceptible to this disease as California-grown Burbanks used in the experiments already described. Early Ohio was apparently most susceptible, in that 10 potatoes rotted out of 11 inoculated. The other varieties seemed somewhat more resistant to this disease.

Inoculations were also made, using potatoes of undetermined varieties purchased in the Washington markets. A fair percentage of these inoculations were successful in all cases. It would seem, then, that susceptibility to this disease is not necessarily confined to potatoes grown on the peat lands of California.

Another series of inoculation experiments was carried out to ascertain what temperatures were most favorable for the growth of the fungus in the potato and at what temperatures no infection would result from an inoculation. In these experiments inoculated potatoes were kept at seven different temperatures, varying in 5-degree intervals from 5° to 35° C. Seventy potatoes of the Burbank variety were used. Forty of these potatoes, those intended for the lower temperatures, were kept in the ice box at about 10° C. for 24 hours before inoculation, so that their temperature at the time of inoculation would be more nearly that at which they were to be maintained during the experiments. For the inoculations

subcultures from five separate isolations of the fungus were used, and 14 potatoes were inoculated from subcultures from each isolation, 2 for each of the constant-temperature chambers. They were maintained at constant temperatures for one week and were then removed and examined. The results of these experiments are shown in Table II.

TABLE II.—Results of experiments in which inoculated potatoes were maintained at constant temperatures, 10 Burbank potatoes in each chamber, maintained at constant temperature for one week. All sound potatoes were then placed in the 30° chamber for three days

Temperature.	Number of tubers showing infection in one week.	Number of tubers which did not show evidences of infection in chambers originally used but which were rotted three days after removal to 30° chamber.	Total number of rotted potatoes.
°C.			
5.....	0	7	7
10.....	3	2	5
15.....	7	2	8
20.....	8	0	8
25.....	9	0	9
30.....	10	0	10
35.....	10	0	10

The results given in Table II show that a higher percentage of inoculated potatoes are rotted at temperatures near the optimum for growth of the fungus in artificial culture media than at the lower temperatures. It is evident, however, that temperatures near this optimum are not necessary for infection. As was to be expected, no rot was produced while the inoculated potatoes were maintained at 5° C., but when these potatoes were moved from this chamber to the incubator maintained at 30°, 70 per cent of them were rotted in three days. The growth of the fungus is apparently inhibited at the low temperature, but begins as soon as the temperature is raised. The lowest total amount of rot was in the potatoes maintained at 10° for the week. In this case 50 per cent of the inoculated potatoes rotted. The growth of the fungus in the potato is slower at the lower temperatures, 10° and 15°, than at the higher temperatures, as was found to be the case with this fungus on artificial-culture media.

It is evident from these experiments in which *P. dcbaryanum* was isolated from 49 diseased tubers, inoculations made from 30 of these isolations into healthy tubers, the disease produced, and the fungus subsequently reisolated that this fungus is frequently present in potatoes affected with leak and that when inoculated into the tubers, it causes this rot.

GROWTH OF *PYTHIUM DEBARYANUM* IN THE TUBER

The rate of growth of the fungus in the potato was approximately determined. A Green Mountain potato which had been inoculated in the usual way and allowed to remain at 30° C. for 67 hours was sliced open. The fungus was found to have penetrated to a depth of 4 cm. from the point of inoculation during this time. The average diameter of the cell of the potato, obtained by measuring a large number of cells, was found to be 138.7 $\mu$ . By calculation the fungus must have passed through approximately 288 cells in 67 hours, or at the rate of 1 cell every 14 minutes. This calculation does not take into account the period of readjustment of the fungus before it begins to grow into the tissue of the potato, which is probably appreciable.

Portions of a potato tuber which had been rotted with *P. debaryanum* were killed, embedded in paraffin, sectioned, and stained.<sup>1</sup> Examination of these sections showed that the mycelium was distributed quite generally throughout the tissue of the host. It usually passes directly through the cell wall (fig. 1, *a*) and through the lumen of the cell, though it was found occasionally between the cells. It branches frequently. Where the hypha of the fungus passes through the cell wall, it is markedly constricted (fig. 1, *a*). Ward (16), in his work on this fungus, also observed that the opening made in the cell wall was smaller than the mean diameter of the fungus hyphæ. Rosénbaum (12) shows the same relation between cell wall of host plant and fungus hypha in his work with *Phytophthora cactorum* on ginseng.

## INFECTION OF POTATOES FROM SOIL

It was mentioned earlier in this paper that the disease was observed only in potatoes which had been wounded. In inoculation experiments it was never possible to cause the disease without first breaking the skin of the potato. The wounds observed in the rotting potatoes in the field studies had been made when the potatoes were harvested, which leads to the conclusion that the organisms causing the leak are probably present in the soil and are introduced into the freshly wounded potato in digging. To obtain evidence on this point, Petri-dish cultures on corn-meal agar were made from samples of the peat soils from various parts of the delta potato region. *P. debaryanum* was found in every case. Inoculations were made by inserting some of the soil into holes in the tubers and in about 50 per cent of the cases the tubers were rotted. *P. debaryanum* was isolated from the rotted tubers.

Field tests were made on the effect of wounding the potatoes in digging. In these experiments seven sacks, or about 12 bushels, of potatoes were harvested. The work was done rather carelessly so that many tubers were injured with the digging forks. The sound potatoes were

<sup>1</sup> The writer is indebted to Mr. Charles S. Ridgway, of the Office of Tobacco Investigations, for the making and staining of these slides. They were stained in methylene blue-eosin combination which leaves the fungus hyphæ bright blue and the cell walls of the host plant red.

sorted out and sacked separately, and all the potatoes were stored in sacks in a warehouse under about the usual commercial conditions. The potatoes were sorted four days later and 65 diseased tubers were found, all of which had been wounded. They were sorted a second time eight days after digging and 52 more rotten tubers were found. None of the unwounded potatoes showed evidences of the disease at any time, and no more of the wounded tubers were rotten when they were sorted for the last time 15 days after harvesting. Transfers were made from some of these rotten tubers to corn-meal agar slants and *P. debaryanum* was obtained in all these cultures. It is evident that this fungus is generally present in these peat soils, that inoculations may be made by inserting some of the soil in wounds in the tubers, and that potatoes wounded in digging frequently become infected. Unwounded tubers are apparently not affected with this disease. It would seem probable from these experiments that more care in harvesting and sorting out of potatoes injured in digging would decrease the losses from this disease.

#### OTHER ROTTS SOMETIMES MISTAKEN FOR LEAK

It is quite possible that tuber-rotts produced by other fungi may be mistaken for potato leak. Two species of *Fusarium*, *F. radicum* Wollenw. and *F. oxysporum* Schlecht., which produce tuber-rotts of the potato are quite common in the San Joaquin potato region. Carpenter (5) has shown that either one or the other of these fungi is usually present in the jelly-end rot of potato tubers. He has also obtained *F. radicum* from specimens of rotten potatoes from San Joaquin County, California, which were supposed to be affected with leak. The present writer has found rotten tubers in consignments of potatoes from California which had stood in the laboratory for a few weeks. These potatoes were apparently sound upon arrival, with the exception of a few which had small rotten spots in the stem end. At the end of a few weeks some of the tubers were entirely rotten and very much resembled those in the advanced stages of leak. *F. radicum* was obtained from several such specimens. Neither *Pythium debaryanum* nor *Rhizopus nigricans* was ever obtained. Potato dealers at Stockton and potato growers say that the leak may develop after the potatoes have been in storage for a time and sometimes after they have been sorted. Under such conditions the rot is apparently not due to *P. debaryanum* nor *R. nigricans*, but to some other organism, probably a species of *Fusarium* in many instances, as in these experiments rotts caused by *P. debaryanum* or *R. nigricans* were usually evident in three or four days. If an inoculated potato was sound at the end of a week it was not infected and the potato would remain sound indefinitely. The experiments in which potatoes were kept at low temperatures are, of course, excepted. It seems quite probable then that potatoes affected with rotts caused by *Fusarium* spp. are sometimes confused with those affected with leak.

## DISCUSSION OF RESULTS

It is evident from the experiments described in this paper and from the work that has been done heretofore that *R. nigricans* rots potato tubers. That it is the cause of a rot of potatoes under field and warehouse conditions has been shown by Orton (10). From the experiments carried out in this study, however, it seems that potato leak is most commonly caused by *P. debaryanum*. At least this seems to have been the case during the season of 1915.

When inoculated into potatoes, both fungi rot the tubers either very rapidly or not at all. It seems that if the disease is not well advanced in a week at 30° C. the potato is not infected. The rots produced by these fungi have practically the same general appearance.

The parasitism of *P. debaryanum* on seedlings of various plants is too well known to require discussion here. That it should be the cause of a potato disease of considerable importance is not surprising when the work of earlier writers is taken into account. Sadebeck (14), in 1875, reported the discovery of a species of *Pythium* parasitic upon potato plants near Coblenz. He considered the fungus to be *P. equiseti* Sadebeck. He mentions finding it on various parts of the plants. That *P. equiseti* was identical with *P. debaryanum* was later pointed out by De Bary (2). De Bary in some of his experiments grew *P. debaryanum* on living potato tubers. Ward (16) also cultivated it on this host and considered potatoes ". . . a very good medium for the cultivation of the fungus." Edson (6) recently obtained this fungus from rotten potato tubers. No one seems to have succeeded in inoculating any part of the potato plant except the tuber with this organism.

That this fungus should cause so much damage to potatoes in the San Joaquin delta region is probably largely due to the conditions and methods of handling the potatoes in that section. As has been said, the potatoes are dug with forks, and many are wounded in the process. Potatoes with branches, or "knobs," are quite common, and these branches are usually broken off in harvesting, if the potato is of marketable size, and the main tuber retained. Perhaps the broken surface of the tuber is rubbed in the soil, "to dry it." That these are excellent methods for inoculating potatoes with *P. debaryanum* has been shown. The potatoes are sacked as soon as dug. They may then stand in the sun for some hours before they are hauled to the car or boat landing for shipment. In the car or on the boat the sacks are usually piled up. The humidity among these tubers is, of course, high because of the high rate of transpiration. This, together with the relatively high temperature, offers good conditions for the development of any parasitic fungus, such as *P. debaryanum*, with which the tubers may have been inoculated. It is quite possible that the leak of potatoes would have been reported from other localities where either *R. nigricans* or *P. debaryanum* are common in the

soils if the methods of harvesting and handling and the temperature conditions were as favorable for the development of these parasites as they are in the delta region of the San Joaquin River.

It is considered by the potato growers of this region that the disease is much more common in hot weather. In these experiments it was shown that the optimum temperature for growth of the fungus is high (between 30° and 35° C.) and that the fungus infects the potatoes more readily at temperatures near this optimum. At the lower temperatures the percentage of infection is not so high, and the growth of the fungus is retarded or, as in the case of the experiments at 5°, inhibited while the potatoes remained at that temperature. It would seem then that lowering the temperatures of the cars and storage warehouse might retard the development of the disease, but that the infected potatoes would rot as soon as the temperature was raised. From the data now at hand, icing the cars and cold storage of the potatoes would seem to be of doubtful value as control measures. The control of the disease seems more likely to lie along the lines of better methods of harvesting and handling, as Orton suggested (10), and a careful sorting out of all wounded tubers.

#### CONCLUSIONS

In the work described in this paper the conclusion of Orton that *Rhizopus nigricans* Ehrenb. can cause a rot of potatoes has been corroborated. This fungus was not, however, isolated in the field experiments from tubers affected with leak. A fungus was obtained 49 times in 61 attempts. The cultures were made from a different tuber each time. A study was made of the fungus and it was found to be *Pythium debaryanum* Hesse. In inoculation experiments this fungus produced a rot typical in all appearances to the potato leak, and was readily reisolated from the diseased tuber. It seems probable that the disease is produced by both *R. nigricans* and *P. debaryanum*. The latter is apparently more frequently the causal organism.

*P. debaryanum* was found in soil samples taken from various parts of the delta potato region. The disease was produced by inserting some of this soil in wounds in the potato tubers and *P. debaryanum* was isolated from these rotted potatoes. Infection apparently takes place in the field by some of this infected soil getting into wounds made in digging. No cases of infection were observed either under field conditions or in the laboratory where the skin of the tuber was unbroken. From the results of these experiments it seems that the disease might be controlled by more care in harvesting and handling the potatoes and a careful sorting out of all wounded tubers.

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PLATE XC

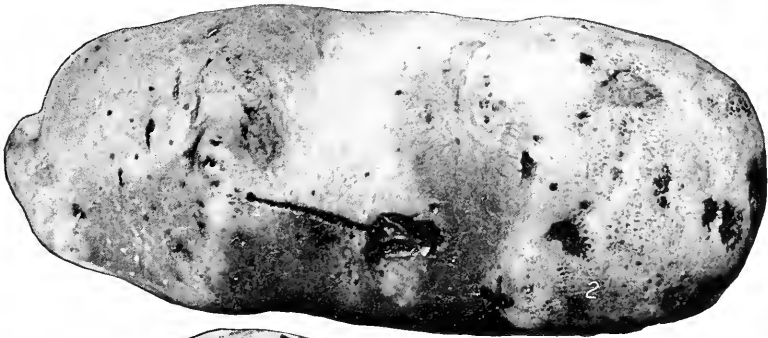
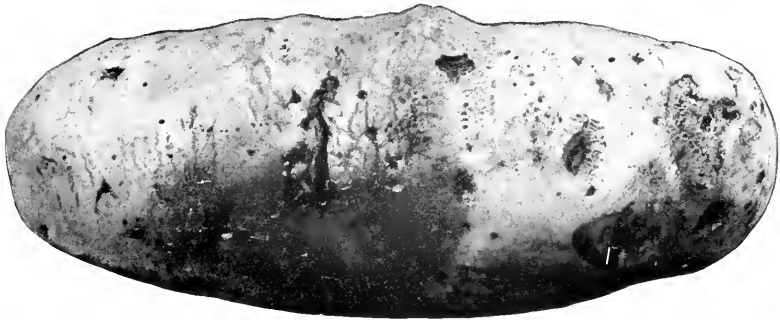
Potatoes affected with potato leak:

Fig. 1, 2.—Natural infection from fork wound; photographed by Dr. W. A. Orton.

Fig. 3.—Rot produced by inoculation with *Pythium debaryanum*.

Fig. 4.—Rot produced by inoculation with *Rhizopus nigricans*. Inoculation made by Mrs. Tillotson.







## DIGESTIBILITY OF HARD PALATES OF CATTLE

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The so-called "hard palates," which are taken from the roof of the mouth of beef animals, have not in the past been utilized to any extent as food. They contain very little muscular tissue, such as is characteristic of meats in general, and possess a ribbed outer surface that is black or white in color, very rough, and of an unattractive appearance.

The microscopic examination of the structural constituents of hard palates of cattle reveals a stratified layer of epithelium which is in a state of cornification. The extent of this layer is possibly one-sixteenth of the entire thickness. The connective tissue portion of the mucous membrane consists of a dense feltwork of white fibrous tissue arranged in dense interlacing bundles; the individual fibers of the bundles, comprising about 60 per cent, are matted together as closely as in tendon tissue or sinews and are interwoven with about 20 per cent of elastic (erectile) fibers, 10 per cent of involuntary muscle, and about 10 per cent of looser fibrous tissue attaching the mucous membrane to the periosteum. This looser tissue contains a small amount of fat and very few glands.

A chemical examination of hard palates showed that when freshly procured they have the following composition: Water, 71.0 per cent; protein (N $\times$ 6.25), 22.2 per cent (or protein by difference, 16.6 per cent); fat, 11.8 per cent; and ash, 0.6 per cent. The high protein content suggested that this material might be of value for food. Since little, if any, experimental evidence is available regarding the thoroughness of digestion of such tissue when eaten in quantity, a number of experiments were undertaken at the suggestion of the Bureau of Animal Industry to determine the digestibility of hard palates by human subjects. This means for practical purposes the digestibility of the nitrogenous material present, since the proportion of fat supplied by the cooked hard palates is small.

### COOKING HARD PALATES

The material for study was obtained from a local abattoir and supplied to the Office of Home Economics by the Bureau of Animal Industry. Before the digestion experiments could be undertaken, it was necessary to find some way of cooking and serving the hard palates which would make it possible to eat them in quantity. At first the attempt was made to put the raw material through an ordinary household meat cutter with the idea that it might then be fried in small cakes, like Hamburg steak, but the material was so firm and tough that it

could not be minced in this way. Accordingly it was decided to cook the palates before trying to mince them, and tests showed that after boiling for two or three hours they could be easily minced with a meat cutter and that so prepared the texture as well as the flavor was not disagreeable, particularly if the palates were combined with other food materials.

The average composition of the cooked palates was found to be as follows: Water, 71.1 per cent; protein ( $N \times 6.25$ ), 21.8 per cent (or protein by difference, 22.3 per cent); fat, 6.3 per cent; and ash, 0.3 per cent. The material used for analysis weighed before cooking  $15\frac{1}{2}$  ounces and after cooking 14 ounces, the total loss therefore being only  $1\frac{1}{2}$  ounces. As will be noted by referring to the percentage composition of the raw material, the boiled palates had, in round numbers, only one-half the fat, one-half the ash, and nine-tenths the protein content of the fresh material. As found by analysis, 50.0 per cent of the ash, 46.6 per cent of the fat, and 11.3 per cent of the protein originally present were removed by cooking. In general, the observed effects are in accord with Grindley's observations<sup>1</sup> that, except for a lowered fat and ash content and the removal of some soluble nitrogenous material, cooked meat has very much the same proximate composition as it has raw.

The water in which the palates were boiled did not look at all like that in which beef is cooked, but was white in color and not unlike milk in appearance. The character of the nitrogenous constituents present was not studied in detail, but preliminary tests indicated that gelatin predominated, with traces of coagulable albumin, globulin, and primary proteoses.

Some attention was given to the hard palate fat which floated to the top of the liquor in which the palates were boiled. This hardened on cooling and was purified by remelting several times to remove the sediment. The product had a deep-yellow color, a mild flavor, and an appearance suggesting butter, though rather more granular. It was found to have a melting point of  $34^{\circ}$  C., an iodine number of 52.53, and a refractive index of 1.4586.<sup>2</sup> The amount obtained was not sufficient for further study.

The cooked palates had a mild and not unpleasant flavor and in appearance resembled cooked gristle or connective tissue rather than lean meat, this resemblance being noticeable even when the material was finely ground. It was apparent that the cooked palates would be much more acceptable as the principal constituent of the experimental ration if prepared in some savory form, and meat cakes and meat loaf naturally suggested themselves as possibilities. The meat cakes

<sup>1</sup> Grindley, H. S., and Mojonier, Timothy. Experiments on losses in cooking meat, 1900-1903. U. S. Dept. Agr. Office Exp. Stas. Bul. 141, p. 94. 1904.

<sup>2</sup> Information regarding the structure and composition of the hard palates and the chemical nature of the material extracted during cooking was supplied by the Bureau of Animal Industry.

did not prove satisfactory, having, when thoroughly cooked and well browned, a flavor suggesting that of scorched or burned gristle or bone. On the other hand, meat loaf made according to a common household recipe and containing in addition to the hard palates some flour, butter, and onions, and sweet herb, salt, and pepper as seasoning was found to be satisfactory for the purpose. The flour served to bind the material together so that the loaf would retain its shape and could be sliced without crumbling, while the butter improved both the texture and the flavor.

#### EXPERIMENTAL RATION

Experience has shown that the normal individual eats more heartily of a food material if it forms a part of a mixed ration than if it is the only food served for several successive meals. Accordingly, with the meat loaf made from hard palates, a uniform basal ration simple in character (crackers and butter, boiled potatoes, and tea or coffee with sugar but no milk or cream) was served. A basal ration which obviously contained only a minimum amount of protein was selected, in order that the hard palates might supply the greater part of the protein of the experimental diet. In making a quantity of the meat loaf sufficient for a three-day digestion experiment for four subjects the following quantities were used: Boiled hard palates finely minced, 13½ pounds; flour, 1 pound; butter, ½ pound; onions, 3 of medium size; and seasoning (sage, salt, and pepper to taste).

#### METHODS OF DIGESTION EXPERIMENTS

Four subjects who had gained experience in this type of work in the study of the digestibility of other foods assisted in this investigation. They were young men of medium weight and of good health, moderately active, and sufficiently informed through previous experience to appreciate the importance of observing accuracy in following all directions given them.

As is evident from a consideration of their composition and the amounts eaten, hard palates supplied only a small part of the total fat of the experimental ration and a very little ash. Furthermore, since little, if any, carbohydrate was present in the hard palates, it follows that interest centers on the digestibility of protein, since this is the only food constituent which they provide in quantity.

Experience has shown that it is desirable to supply a food constituent in generous proportions in order that the calculated coefficients of digestibility may not be masked by unavoidable errors incidental to the methods followed. To make sure that the amount of protein eaten was generous, a fairly large allowance of the meat loaf made from hard palates was served at each meal and the subjects were urged to eat all of it. At the same time, as already noted above, only a limited amount of protein was obtainable from other sources.

As regards the experimental details, the methods followed in studying the hard palates were similar to those previously reported with other foods.<sup>1</sup> As no attempt was made to maintain body weight or to approximate a nitrogen equilibrium, the quantity of the entire ration to be eaten was not stipulated. The feces occurring from each experimental period, as indicated by charcoal markers, were collected and dried to remove the water. Samples of foods eaten were retained for analysis and all analyses of foods and feces were made by the methods described by the Association of Official Agricultural Chemists.<sup>2</sup>

In order to determine the digestibility of a single food contained in a mixed diet, it is necessary either to determine the digestibility of the basal ration and to apply the proper correction to the values obtained for the digestibility of the total diet, or to estimate the undigested residue occurring from the various constituents of the diet by means of coefficients previously determined, and to make proper allowance for this undigested material. The latter method has been followed in this instance and the method of estimating the digestibility of the protein of the meat loaf alone is indicated by the following equations:

$$[\text{Weight of protein in potato, crackers, and butter}] \times [\text{Percentage of undigested protein occurring in each}] = [\text{Weight of undigested protein present in feces derived from basal ration}].$$

$$[\text{Total undigested protein in feces}] - [\text{Undigested protein in feces from basal ration}] = [\text{Undigested protein occurring from meat loaf}].$$

$$[(\text{Total protein of meat loaf}) - (\text{Undigested protein from meat loaf})] \div [\text{Total protein of meat loaf}] = [\text{Estimated percentage digestibility of meat loaf alone}].$$

On the basis of determinations by previous investigators the coefficients assumed in these equations for the digestibility of the protein of the potatoes, crackers, and butter are 83 per cent,<sup>3</sup> 93.8 per cent,<sup>4</sup> and 97 per cent,<sup>3</sup> respectively.

In Table I are recorded the essential experimental data of the digestion experiments with hard palates, including the total weight of food eaten, the nutrients furnished, the weight of feces, the undigested nutrients therein, the percentage of the different nutrients digested, and the estimated digestibility of the protein of the meat loaf.

<sup>1</sup> Langworthy, C. F., and Holmes, A. D. Digestibility of some animal fats. U. S. Dept. Agr. Bul. 310, 22 p. 1915.

<sup>2</sup> Wiley, H. W. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted in 1912.

<sup>3</sup> Atwater, W. O., and Bryant, A. P. The availability and fuel value of food materials. *In* Conn. Storrs Agr. Exp. Sta. 12th Ann. Rpt., 1899, p. 104. 1900.

<sup>4</sup> Woods, C. D., and Merrill, L. H. Studies on the digestibility and nutritive value of bread at the Maine agricultural experiment station, 1899-1903. U. S. Dept. Agr. Office Exp. Stas. Bul. 143, p. 33. 1904.

TABLE I.—Results of digestion experiments with hard palates of cattle in a simple mixed diet

Item.	Weight of food.	Water.	Protein.	Fat.	Carbo-hydrates.	Ash.
<b>Experiment 334 (subject H. F. B.):</b>						
Hard palates (in form of meat loaf).....gm..	1,309	749.3	317.4	141.2	71.4	29.7
Potato.....gm..	1,242	937.7	31.1	1.2	259.6	12.4
Crackers.....gm..	670	40.0	56.5	96.5	472.3	4.7
Butter.....gm..	261	28.7	2.6	221.9	.....	7.8
Sugar.....gm..	213	.....	.....	.....	213.0	.....
Total food consumed, gm.....	3,695	1,755.7	407.6	460.8	1,016.3	54.6
Feces.....gm..	136	.....	72.3	18.8	32.1	12.8
Amount utilized.....gm..	.....	.....	335.3	442.0	984.2	41.8
Digestibility of entire ration.....per cent..	.....	.....	82.3	95.9	96.8	76.6
Estimated digestibility of meat loaf.....per cent..	.....	.....	80.0	.....	.....	.....
<b>Experiment 335 (subject D. G. C.):</b>						
Hard palates (in form of meat loaf).....gm..	1,327	759.6	321.8	143.2	72.3	30.1
Potato.....gm..	1,232	930.2	30.8	1.2	257.5	12.3
Crackers.....gm..	661	39.5	55.8	95.2	465.9	4.6
Butter.....gm..	313	34.4	3.1	266.1	.....	9.4
Sugar.....gm..	111	.....	.....	.....	111.0	.....
Total food consumed, gm.....	3,644	1,763.7	411.5	505.7	906.7	56.4
Feces.....gm..	105	.....	54.5	21.0	20.0	9.5
Amount utilized.....gm..	.....	.....	357.0	484.7	886.7	46.9
Digestibility of entire ration.....per cent..	.....	.....	86.8	95.8	97.8	83.2
Estimated digestibility of meat loaf.....per cent..	.....	.....	85.8	.....	.....	.....
<b>Experiment 336 (subject R. L. S.):</b>						
Hard palates (in form of meat loaf).....gm..	1,329	760.7	322.3	143.4	72.4	30.2
Potato.....gm..	1,275	962.6	31.9	1.3	266.5	12.7
Crackers.....gm..	291	17.4	24.6	41.9	205.1	2.0
Butter.....gm..	268	22.9	2.1	176.8	.....	6.2
Sugar.....gm..	69	.....	.....	.....	69.0	.....
Total food consumed, gm.....	3,172	1,763.6	380.9	363.4	613.0	51.1
Feces.....gm..	70	.....	32.8	15.9	13.4	7.9
Amount utilized.....gm..	.....	.....	348.1	347.5	599.6	43.2
Digestibility of entire ration.....per cent..	.....	.....	91.4	95.6	97.8	84.5
Estimated digestibility of meat loaf.....per cent..	.....	.....	92.0	.....	.....	.....

TABLE I.—Results of digestion experiments with hard palates of cattle in a simple mixed diet—Continued

Item.	Weight of food.	Water.	Protein.	Fat.	Carbo-hydrates.	Ash.
Experiment 337 (subject O. E. S.):						
Hard palates (in form of meat loaf).....gm..	1,052	602.2	255.1	113.5	57.3	23.9
Potato.....gm..	1,126	850.1	28.2	1.1	235.3	11.3
Crackers.....gm..	525	31.3	44.3	75.6	370.1	3.7
Butter.....gm..	258	28.4	2.6	219.3	.....	7.7
Sugar.....gm..	467	.....	.....	.....	467.0	.....
Total food consumed, gm.....	3,428	1,512.0	330.2	409.5	1,129.7	46.6
Feces.....gm..	98	.....	41.4	31.7	14.7	10.2
Amount utilized.....gm..	.....	.....	288.8	377.8	1,115.0	36.4
Digestibility of entire ration.....per cent.....	.....	.....	87.5	92.3	98.7	78.1
Estimated digestibility of meat loaf.....per cent.....	.....	.....	86.8	.....	.....	.....
Experiment 342 (subject H. F. B.):						
Hard palates (in form of meat loaf).....gm..	1,312	755.2	336.2	118.5	86.8	15.3
Potato.....gm..	1,216	918.1	30.4	1.2	254.1	12.2
Crackers.....gm..	309	21.3	25.0	41.4	218.8	2.5
Butter.....gm..	107	11.8	1.1	90.9	.....	3.2
Sugar.....gm..	317	.....	.....	.....	317.0	.....
Total food consumed, gm.....	3,261	1,706.4	392.7	252.0	876.7	33.2
Feces.....gm..	97	.....	54.4	14.1	19.9	8.6
Amount utilized.....gm..	.....	.....	338.3	237.9	856.8	24.6
Digestibility of entire ration.....per cent.....	.....	.....	86.1	94.4	97.7	74.1
Estimated digestibility of meat loaf.....per cent.....	.....	.....	85.8	.....	.....	.....
Experiment 343 (subject D. G. G.):						
Hard palates (in form of meat loaf).....gm..	1,473	847.9	377.5	133.0	97.4	17.2
Potato.....gm..	1,176	887.9	29.4	1.2	245.8	11.7
Crackers.....gm..	376	25.9	30.5	50.4	266.2	3.0
Butter.....gm..	288	31.7	2.9	244.8	.....	8.6
Sugar.....gm..	96	.....	.....	.....	96.0	.....
Total food consumed, gm.....	3,409	1,793.4	440.3	429.4	705.4	40.5
Feces.....gm..	93	.....	50.3	15.5	19.6	7.6
Amount utilized.....gm..	.....	.....	390.0	413.9	685.8	32.9
Digestibility of entire ration.....per cent.....	.....	.....	88.6	96.4	97.2	81.2
Estimated digestibility of meat loaf.....per cent.....	.....	.....	88.5	.....	.....	.....



TABLE I.—Results of digestion experiments with hard palates of cattle in a simple mixed diet—Continued

Item.	Weight of food.	Water.	Protein.	Fat.	Carbohy- drates.	Ash.
Experiment 345 (subject O. E. S.):						
Hard palates (in form of meat loaf).....gm..	1, 296	746. 0	332. 1	117. 0	85. 7	15. 2
Potato.....gm..	1, 009	761. 8	25. 2	1. 0	210. 9	10. 1
Crackers.....gm..	345	23. 8	27. 9	46. 2	244. 3	2. 8
Butter.....gm..	104	11. 5	1. 0	88. 4	.....	3. 1
Sugar.....gm..	340	.....	.....	.....	340. 0	.....
Total food consumed, gm.....	3, 094	1, 543. 1	386. 2	252. 6	880. 9	31. 2
Feces.....gm..	98	.....	45. 3	20. 4	22. 1	10. 2
Amount utilized.....gm..	.....	.....	340. 9	232. 2	858. 8	21. 0
Digestibility of entire ration.....per cent..	.....	.....	88. 2	91. 9	97. 5	67. 3
Estimated digestibility of meat loaf.....per cent..	.....	.....	88. 2	.....	.....	.....
Average food consumed per subject per day.gm..	1, 129	563. 7	130. 9	127. 3	291. 8	14. 9

## SUMMARY

Experi- ment No.	Subject.	Digestibility of entire ration (per cent).				Estimated digesti- bility of protein of meat loaf alone (per cent).
		Protein.	Fat.	Carbo- hydrates.	Ash.	
334	H. F. B.....	82. 3	95. 9	96. 8	76. 6	80. 0
335	D. G. G.....	86. 8	95. 8	97. 8	83. 2	86. 8
336	R. L. S.....	91. 4	95. 6	97. 8	84. 5	91. 9
337	O. E. S.....	87. 5	92. 3	98. 7	78. 1	86. 7
342	H. F. B.....	86. 1	94. 4	97. 7	74. 1	85. 8
343	D. G. G.....	88. 6	96. 4	97. 2	81. 2	88. 5
345	O. E. S.....	88. 2	91. 9	97. 5	67. 3	88. 2
	Average.....	87. 3	94. 6	97. 6	77. 9	86. 8

The average amount of food eaten per subject per day was 1,129 gm. which furnished 564 gm. of water, 131 gm. of protein, 127 gm. of fat, 292 gm. of carbohydrates, and 15 gm. of ash. The uniformity of values obtained in the different experiments for the digestibility of the carbohydrates and the close agreement of the average value, 97.6 per cent, with the value, 97 per cent, given for the digestibility of carbohydrates in the ordinary mixed diet<sup>1</sup> would indicate that care had been observed in the collection of the feces. The digestibility of fat is of interest, in that practically all of the fat of the diet was obtained from the butter,

<sup>1</sup>Atwater, W. O. On the digestibility and availability of food materials. *In* Conn. Storrs Agr. Exp. Sta. 14th Ann. Rpt., 1901, p. 245. 1902.

part of which was present as a constituent of the meat loaf and a part as a constituent of the basal ration, supplying in all approximately 125 gm. of fat per subject per day. This was 94.6 per cent assimilated, which for all practical purposes is identical with the digestibility of butter found in a previous investigation,<sup>1</sup> 93.9 per cent.

Inasmuch as the subjects were allowed to eat of the basal ration according to individual preferences, the energy value of the diet was not uniform. It was found, however, that the subjects eating as much as they wished received, on an average, 3,265 Calories daily, calculated from the average daily consumption of protein, fat, and carbohydrates, and the factors<sup>2</sup> commonly used in the determination of fuel values. In view of the fact that over 130 gm. of protein, largely supplied by the meat loaf, and over 3,200 Calories of energy were consumed daily, it is apparent that the ration was eaten with relish.

The digestibility of the total protein of the diet was found to be 87.3 per cent. The meat loaf supplied 82 per cent of the total protein consumed, a much larger proportion than is ordinarily furnished by the meat portion of a meal; consequently, greater accuracy is possible in estimating the digestibility of the protein contained in the meat loaf.

The digestibility of the protein of the meat loaf alone, 86.8 per cent, differs little from the value of the digestibility of the entire ration. This is due partly to the rather complete assimilation of the protein of the basal ration and partly to the relatively small amount of protein derived from this source. The value, 86.8 per cent, represents the digestible protein of the meat loaf, but it should closely approximate that for the protein of the hard palates, since in the preparation of the loaf the proportions used were 13.5 parts of hard palates to 1 part of flour. An allowance may be made for the flour by assuming the protein from this source to be 93.8 per cent<sup>3</sup> digestible. From the results of this investigation, accordingly, it would seem that the protein of hard palates which have been thoroughly cooked is somewhat less thoroughly assimilated than that of the common cuts of meat.<sup>4</sup>

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<sup>1</sup> Langworthy, C. F., and Holmes, A. D. Digestibility of some animal fats. U. S. Dept. Agr. Bul. 310, 22 p. 1915.

<sup>2</sup> Atwater, W. O., and Bryant, A. P. The availability and fuel value of food materials. *In* Conn. Storrs Agr. Exp. Sta. 12th Ann. Rpt. 1899, p. 104. 1900.

<sup>3</sup> Woods, C. D., and Merrill, L. H. Studies on the digestibility and nutritive value of bread at the Maine agricultural experiment station, 1899-1923. U. S. Dept. Agr. Office Exp. Stas. Bul. 143, p. 33. 1904.

<sup>4</sup> Grindley, H. S., Mojonner, Timothy, and Porter, H. C. Studies of the effect of different methods of cooking upon the thoroughness and ease of digestion of meat at the University of Illinois. U. S. Dept. Agr. Office Exp. Stas. Bul. 193, 100 p. 1927.

# SOME PROPERTIES OF THE VIRUS OF THE MOSAIC DISEASE OF TOBACCO

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## INTRODUCTION

Several theories have been advanced to explain the physiological origin of the mosaic disease of tobacco (*Nicotiana tabacum*.) Independently, Woods (20)<sup>1</sup> and Heintzel (10) came to the conclusion that oxidizing enzymes are responsible for the disease. Hunger (11) did not accept the enzymic theory of the mosaic disease but considered that unfavorable conditions of growth produced specific toxins within the plant which led to the appearance of the disease. The writer (1) has secured data which do not lend support to the physiological origin of the disease, but indicate that it is dependent upon specific infection.

Further studies of the properties of the expressed sap of mosaic plants, termed the "virus" of the disease, have thrown considerable light on the nature of the infective principle and its relation to some of the enzymic properties of the sap of diseased plants.

Woods (20) and other workers following him have attributed the origin of the mosaic disease to oxidases and peroxidases existing normally in healthy tobacco plants. Since it is a question of fundamental importance to determine whether or not such enzymes are the primary cause of the disease, their relation to infection has been more fully investigated. All data at hand indicate that infection does not depend upon the presence of oxidases or peroxidases, but upon an infective principle which is not a normal constituent of the sap of healthy plants. These conclusions rest upon the fact that methods have been found by which the infective principle may be separated from the oxidases and peroxidases present in the sap of mosaic plants, as shown in the experimental work.

## FILTRATION EXPERIMENTS WITH THE VIRUS OF THE DISEASE

### FILTRATION THROUGH THE LIVINGSTONE ATMOMETER POROUS CUP

Earlier investigators have shown that the virus of the mosaic disease of tobacco passes through the Berkefeld (normal) filter without losing its infectious properties. The writer's experiments substantiate these results, as shown in Table I, although there is strong indication that the virus becomes attenuated and is less infectious when filtered in this

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<sup>1</sup> Reference is made by number to "Literature cited," p. 673-674.

way. The writer has been unable to obtain the finer pored Berkefeld or Pasteur-Chamberland bougies of the same type because of the European war. By using the Livingstone atmometer porous cup, however, a method of filtration has been devised which has given very interesting results. The construction of this apparatus is shown in Plate XCI. The extracted sap is first filtered through filter paper to remove all suspended material. The clear dark-amber solution is then filtered through the porous cup under reduced pressure (approximately 3 inches of mercury). After passing through the atmometer, the virus has completely lost its infectious properties, yet an intense peroxidase reaction is given with guaiac and hydrogen peroxid.<sup>1</sup>

TABLE I.—*Infectivity of the mosaic virus after it has been filtered through the Livingstone atmometer porous cup in 1915, 10 Connecticut Broadleaf plants having been used in each test*

Virus used.	Enzymic reactions before treatment.	Treatment.	Enzymic reactions after treatment.	Date of inoculation.	Result.
Virus X <sup>16</sup> .....	Intense peroxidase, intense catalase.	Untreated.....	Intense peroxidase, intense catalase.	Nov. 9	8 mosaic.
Do.....	.....do.....	Filtered through paper only.	.....do.....	.....do.....	Do.
Do.....	.....do.....	Filtered through atmometer.	.....do.....	.....do.....	All healthy.
Tap water (control).	.....do.....	.....do.....	.....do.....	.....do.....	Do.
Virus X <sup>23</sup> .....	Intense peroxidase, weak catalase.	Filtered through paper only.	Intense peroxidase, weak catalase.	Nov. 24	4 mosaic.
Virus X <sup>23</sup> , portion A.	.....do.....	Filtered through atmometer, taken after filtering 2 hours.	Intense peroxidase	.....do.....	All healthy.
Virus X <sup>23</sup> , portion B.	.....do.....	Filtered through atmometer, taken after filtering 1 hour.	.....do.....	.....do.....	Do.
Virus X <sup>23</sup> , portion C.	.....do.....	.....do.....	.....do.....	.....do.....	Do.
Virus X <sup>23</sup> , portion D.	.....do.....	.....do.....	.....do.....	.....do.....	Do.
Tap water (control).	.....do.....	.....do.....	.....do.....	.....do.....	Do.

#### FILTRATION THROUGH POWDERED TALC

Numerous experiments have shown that the infective principle of the mosaic disease of tobacco may be completely removed by filtering the virus through powdered talc.

In these experiments (Table II) Hirsch's porcelain funnel, having a diameter of 9 to 10 cm. and furnished with a stationary perforated disk, was used. A disk of hard filter paper was placed over this disk to retain

<sup>1</sup> Woods used the guaiac and guaiac hydrogen-peroxid tests giving the blue coloration for the determination of oxidases and peroxidases in the extracted sap of tobacco plants. Since the oxidase theory as expressed by Woods was based upon results secured with these tests, the same tests were used in the writer's experiments. The terms "intense," "strong," "weak," etc., have been used to designate the relative intensity of the blue coloration. An "intense" peroxidase reaction is one giving at once an intense indigo blue. The term "strong" indicates that the blue coloration is not as deep, and appears more slowly. The term "weak" denotes a light-blue coloration.

the talc, which was mixed with water and poured upon the paper. This filter must be very carefully made, as bubbles and cracks which may form as a result of shrinkage due to drying during the process of making render the results unreliable. Filtration was accomplished by means of a reduced pressure of approximately 3 inches of mercury.

TABLE II.—*Infectivity of mosaic virus after having been filtered through different thicknesses of powdered talc, U. S. P., in 1915*

Virus used.	Peroxidase reactions before filtering.	Material used for inoculation.	Peroxidase reactions after filtering.	Date of inoculation.	Number of plants inoculated.	Results.
X <sup>100</sup> .....	Intense..	Filtrate from 1-inch talc.....	Intense..	Mar. 6	20	All healthy.
Do.....	do.....	Residue on surface of above talc.....	do.....	do.....	10	7 mosaic.
Tap water (control).	.....	.....	.....	do.....	10	All healthy.
X <sup>100</sup> .....	Intense..	Filtrate from 1-inch talc.....	Intense..	Mar. 21	20	Do.
Do.....	do.....	Residue on surface of above talc.....	do.....	do.....	10	8 mosaic.
Tap water (control).	.....	.....	.....	do.....	10	All healthy.
X <sup>99</sup> .....	Intense..	First portion of filtrate from 1¼-inch talc; color light amber.	None....	Apr. 29	10	Do.
Do.....	do.....	Second portion of above filtrate 2 hours later; color darker.	do.....	do.....	20	Do.
Do.....	do.....	Residue on surface of above talc.....	Intense..	do.....	20	19 mosaic.
X <sup>97</sup> .....	do.....	Filtrate from 7/8-inch talc.....	do.....	do.....	10	7 mosaic.
Do.....	do.....	do.....	do.....	do.....	10	Do.
Do.....	do.....	Filtrate from 2-inch talc.....	do.....	do.....	10	Do.
Tap water (control).	.....	.....	.....	do.....	10	Do.
X <sup>96</sup> .....	Intense..	First portion of filtrate from 1½ inch talc; color light.	None....	Apr. 30	10	Do.
Do.....	do.....	Second portion of above filtrate; color very dark.	Intense..	do.....	10	Do.
Do.....	do.....	Residue on surface of above talc.....	do.....	do.....	10	10 mosaic.
X <sup>98</sup> .....	do.....	First portion of filtrate from 1½-inch talc; color light.	None....	do.....	10	All healthy.
Do.....	do.....	Residue on surface of above talc.....	Strong..	do.....	20	9 mosaic.
X <sup>100</sup> .....	do.....	Filtrate from 1-inch talc.....	do.....	May 4	10	All healthy.
Do.....	do.....	Residue on surface of above talc.....	do.....	do.....	10	8 mosaic.

Experiments (Table III) have shown that thick layers of talc, by adsorption, remove all the peroxidase from the pure virus. If no peroxidase reactions are shown, or if these reactions have been appreciably weakened, such filtrates have always lost their infectious properties. By reducing the amounts of talc, however, the peroxidase content may be increased until limits are reached beyond which the infective principle also passes into the filtrates. In some of the writer's filtration tests the first portions of the filtrate, giving intense peroxidase reactions, possessed no infectious properties, while the last portions contained the infectious principle of the disease. By using known quantities of powdered talc and constant quantities of different concentrations of virus, it is readily shown that the peroxidase content of the filtrates is not definitely related to infectivity. The Hirsch porcelain funnel was used as in preceding talc filtration tests. The virus was first filtered through paper to remove suspended material. All dilutions were made with distilled water. Filtration was accomplished by means of a reduced pressure of approximately 3 inches of mercury.

TABLE III.—*Infectivity of mosaic virus after filtering constant quantities of different concentrations through weighed amounts of powdered talc, U. S. P.*

Strength of virus after dilution with distilled water.	Peroxidase reactions before filtering.	Quantity of talc.	Time required to filter 50 c. c. of solution.	Peroxidase reactions after filtering.	Number of plants inoculated.	Results.
50 c. c. of undiluted virus.....	Intense...	Gm. ( <sup>a</sup> )	Minutes.	Intense...	20	15 mosaic.
Do.....	do.....	36	30	Weak....	10	All healthy.
Do.....	do.....	18	20	Intense...	10	Do.
50 c. c. of 60 per cent virus.....	do.....	18	9	Strong....	10	Do.
50 c. c. of 40 per cent virus.....	do.....	( <sup>c</sup> )	.....	do.....	10	9 mosaic.
Do.....	Strong....	18	6	None.....	10	All healthy.
50 c. c. of 20 per cent virus.....	do.....	18	( <sup>b</sup> )	do.....	10	Do.
50 c. c. of 4 per cent virus.....	do.....	18	do.....	do.....	10	Do.
50 c. c. of 56 per cent virus.....	Intense...	9	9	Strong....	10	Do.
50 c. c. of 24 per cent virus.....	Strong....	9	( <sup>b</sup> )	Weak....	10	Do.
50 c. c. of 20 per cent virus.....	do.....	9	5	Very weak	10	Do.
50 c. c. of 4 per cent virus.....	do.....	9	4	None.....	10	Do.
50 c. c. of undiluted virus.....	Intense...	9	15	Intense...	10	Do.
50 c. c. of 10 per cent virus.....	Strong....	4.5	5	Weak....	10	Do.
50 c. c. of 4 per cent virus.....	do.....	4.5	5	None.....	10	Do.
50 c. c. of 5 per cent virus.....	do.....	2.3	2	Good....	10	Do.
50 c. c. of 3 per cent virus.....	do.....	2.3	1½	None.....	10	Do.
50 c. c. of 2 per cent virus.....	Good.....	1.1	1½	do.....	10	Do.

<sup>a</sup> Paper only.<sup>b</sup> Not timed.

These results indicate that the infective agents producing the mosaic disease are readily arrested by means of the talc filter. Likewise, it is shown that filtered solutions giving intense peroxidase reactions are no longer infectious.

#### PRECIPITATION OF THE VIRUS WITH ETHYL ALCOHOL

Experiments have shown that the infective properties of the mosaic disease are quickly destroyed by the higher strengths of ethyl alcohol. Although a strength of 80 per cent appears to destroy the infective properties of the virus in half an hour, the peroxidase continues to give strong reactions with guaiac and hydrogen peroxid. In various experiments the enzymes have been precipitated in solutions of virus of sufficient alcoholic strength to destroy its infective properties. The virus was first passed through filter paper to remove all material in suspension. This gave a clear, dark, wine-colored solution, which was then made up to different alcoholic strengths with absolute alcohol.

In the first test virus X<sup>16</sup>, giving intense peroxidase and catalase reactions, was used. On November 1, 1915, 200 c. c. of this virus were made up to a 75 per cent alcoholic strength with absolute alcohol. On November 2 the solution was filtered and the precipitate air dried to remove the alcohol. On November 3 the residue remaining was taken up with 50 c. c. of distilled water. Of the filtrate, 750 c. c. were then evaporated to dryness at room temperature, from November 2 to November 4. This filtrate contained neither peroxidase nor catalase. After evaporation, the amber-colored residue, which is readily soluble, was taken up with distilled water.

In a second test the highly infectious virus X<sup>20</sup>, giving intense peroxidase reactions but no reaction for catalase, was used, and 400 c. c. of this virus

were made up to an alcoholic strength of 80 per cent with absolute alcohol. This solution was prepared on November 6, 1915, and allowed to stand until November 8, when the precipitate was collected by filtration and evaporated to dryness at room temperatures. This residue was taken up with 100 c. c. of distilled water. The original filtrate was also tested for peroxidase and likewise by inoculation.

Since earlier experiments have shown that the infective principle is not destroyed in alcoholic strengths of 45 to 50 per cent for several days, precipitation tests were also made with these strengths. Of virus X<sup>20</sup>, used in the preceding test, 160 c. c. were made up to a 50 per cent alcoholic solution with absolute alcohol on November 6, 1915. A portion of the supernatant, clear solution was then siphoned off very carefully without disturbing the heavy, flocculent precipitate below. The precipitate was then collected on filter paper and freed from alcohol at room temperatures on November 8 to November 10. This residue was then taken up with 100 c. c. of distilled water (Table IV).

TABLE IV.—*Infectivity of mosaic virus after having been precipitated in 75, 80, 50, and 45 per cent alcoholic solutions*

Virus used.	Alcoholic strengths.	Treatment.	Enzymic reactions after treatment.	Number of plants inoculated.	Result.
X <sup>16</sup> .....	<i>Per cent.</i> 75	Precipitate evaporated dry and taken up with 50 c. c. of water.	Intense peroxidase, intense catalasc.	10	All healthy.
Do.....	75	Filtrate from above evaporated dry and taken up with water.	No peroxidase, no catalase.	10	Do.
Do.....		Original, untreated virus.....	Intense peroxidase, intense catalase.	20	17 mosaic.
Tap water only (control).				10	All healthy.
X <sup>20</sup> .....	80	Precipitate evaporated dry and taken up with 100 c. c. of water.	Intense peroxidase	10	Do.
Do.....	80	Filtrate from above not evaporated.	No peroxidase.....	10	Do.
Do.....		35 c. c. original virus evaporated dry and taken up with 20 c. c. of water.	Intense peroxidase, no catalase.	10	5 mosaic.
Tap water only (control).				10	All healthy.
X <sup>25</sup> .....	50	Precipitate evaporated dry and taken up with 100 c. c. of water.	Strong peroxidase.	10	9 mosaic.
Do.....	50	Supernatant solution siphoned off from above precipitate and not evaporated.	.....do.....	10	All healthy.
Do.....	45	Precipitate not filtered or evaporated.	Intense peroxidase	10	9 mosaic.
Do.....	45	Second portion of unfiltered precipitate, 15 c. c. diluted with 15 c. c. of water.	.....do.....	10	10 mosaic.
Do.....	45	200 c. c. of precipitate evaporated dry and taken up with 150 c. c. of water.	.....do.....	10	Do.
Do.....	45	Supernatant solution siphoned off, but not filtered or evaporated.	Strong peroxidase.	10	All healthy.
Do.....	45	Supernatant solution siphoned off and filtered through paper only.	.....do.....	10	Do.
Do.....	45	15 c. c. of unfiltered supernatant solution diluted with 15 c. c. of water.	.....do.....	10	Do.
Do.....	45	1,000 c. c. of filtered, supernatant solution evaporated dry and taken up with 400 c. c. of water.	.....do.....	10	Do.
Do.....		Original virus untreated, but diluted to 1 part of virus in 500 parts of water.	No peroxidase.....	10	8 mosaic.
Tap water only (control).				10	All healthy.

In the next experiment a 45 per cent alcoholic solution of virus was made up with virus X<sup>23</sup> as follows: 825 c. c. of this virus, which had been previously filtered through filter paper, were shaken with 675 c. c. of absolute alcohol on January 14, 1916. On January 15 the clear, supernatant solution was siphoned off. A portion of this was filtered through hard filter paper and a second portion was left unfiltered. Of the unfiltered portion 15 c. c. were also diluted with 15 c. c. of distilled water. On January 15, 1,000 c. c. of the supernatant solution which had been filtered through paper were set aside in a large, shallow dish to evaporate. On January 20 the dry residue was taken up with 400 c. c. of distilled water, which gave a somewhat stronger concentration than the original virus. (See Table IV.)

After decanting off as much of the supernatant solution as possible, the heavy, semiliquid precipitate, or sludge, was treated as follows: A portion was left unfiltered; a second portion was diluted by adding 15 c. c. of distilled water to 15 c. c. of the sludge. In addition to this, 200 c. c. of the sludge were placed in a beaker to evaporate to dryness at room temperatures on January 15. The dry residue was taken up with 150 c. c. of distilled water on January 17. Inoculation tests were now made with the virus after undergoing the various treatments outlined above in connection with precipitations with ethyl alcohol.

From Table IV it will be seen that the infective principle of the virus has been completely destroyed in the 75 per cent and 80 per cent alcoholic solutions, although the precipitates continued to give intense reactions for peroxidase. In these strengths precipitation of the peroxidase was complete, as the supernatant solutions gave no reaction for this enzym.

In the 45 per cent and 50 per cent alcoholic solutions, the infective principle was not appreciably injured. The infective agent, however, appears to have been carried down with the heavy, flocculent precipitates, leaving the supernatant solutions free from infective properties. Owing to the fact that the peroxidase remained in solution, the supernatant solutions continued to give strong peroxidase reactions. According to Chodat and Bach (7), the oxygenase in the sap of a species of *Lactarius* could be largely precipitated by 40 per cent alcohol, while the peroxidase remained in solution.

The writer's experiments indicate that concentrated solutions of peroxidase precipitated by strong alcohol from the sap of mosaic plants will not produce infection in healthy plants. Furthermore, the writer has carried out successive re-solutions in water and re-precipitations with alcohol in order to obtain purer solutions of peroxidase. Such solutions, however, have never produced infection, although giving intense reactions for peroxidase and in some instances for catalase.



## TREATMENT OF THE VIRUS WITH HYDROGEN PEROXID

Experiments have shown that certain quantities of hydrogen peroxid (U. S. P., 3.10 per cent) may be added to the virus of the mosaic disease without destroying its infectious properties. By treating the virus with different quantities of hydrogen peroxid, it is possible to find concentrations which destroy the peroxidase and at the same time leave little or no free hydrogen peroxid in the solution. Schönbein (19, p. 474) and likewise Bach and Chodat (2, p. 603) have observed that while peroxidase activates small amounts of hydrogen peroxid, large amounts of hydrogen peroxid destroy the peroxidase (Table V).

These results show that hydrogen peroxid may destroy the peroxidase in the virus without destroying its infectious properties. Although such solutions no longer give peroxidase reactions, they may retain their infectious properties for a long time. If the quantity of hydrogen peroxid is considerably increased beyond that concentration which is sufficient to destroy all the peroxidase, hydrogen peroxid remains in excess in the solution and the virus sooner or later loses its property of infection.

Chodat (6, p. 642-645) and other investigators have shown the definite relations existing between peroxidase, hydrogen peroxid, and the oxidation products. It has been shown that for constant quantities of peroxidase, the oxidation products increase directly with the amount of hydrogen peroxid present, within certain limits, until all the peroxidase is combined or used up.

The quantity of hydrogen peroxid required to destroy the peroxidase varies greatly, depending upon the composition of the virus. If the virus evolves little or no oxygen upon the addition of hydrogen peroxid, a very small quantity of this reagent destroys the peroxidase.

From Table V it will be seen that a very small quantity of hydrogen peroxid (3.1 per cent, U. S. P.) destroyed the peroxidase in virus X<sup>20</sup>. As the quantities of hydrogen peroxid were increased, a point was reached where the excess was sufficient to kill the infective principle of the virus. If a considerable excess is present in solutions of virus for any length of time, such solutions lose their green or brown color and become pale or almost as clear as water in some instances. With the addition of 2 c. c. of hydrogen peroxid to 23 c. c. of virus X<sup>20</sup>, a small excess of hydrogen peroxid was noticeable for several days, but this later disappeared. It has been observed by Bach and Chodat (3, p. 173) that if a mixture of peroxidase and hydrogen peroxid is allowed to stand for some time, both disappear from the solution by mutual interaction and destruction.

TABLE V.—Effect of hydrogen peroxid upon the infectivity of virus secured from different sources in 1915, 10 plants having been used in each test

Virus used.	Date extracted.	Enzymic reactions before treatment.	Treatment.	Enzymic reactions after treatment.	Date inoculated.	Result.
X <sup>1</sup>	.....	Strong peroxidase.....	15 c. c. virus+5 c. c. H <sub>2</sub> O <sub>2</sub> , prepared Jan. 2	{No peroxidase, Jan. 5. {No peroxidase, May 29.	{Jan. 5 {May 29	{8 mosaic. {to mosaic. Do.
Do.	.....	do.	17 c. c. virus+3 c. c. H <sub>2</sub> O <sub>2</sub> , prepared Jan. 2	{Fair peroxidase, Jan. 5. {Weak peroxidase, Jan. 12.	{Jan. 5 {Jan. 5	{Do. {Do.
Do.	.....	do.	18 c. c. virus+2 c. c. H <sub>2</sub> O <sub>2</sub> , prepared Jan. 2	{No peroxidase, May 29.	{May 29	{3 mosaic.
Do.	.....	do.	19.5 c. c. virus+0.5 c. c. H <sub>2</sub> O <sub>2</sub> , prepared Jan. 2	{Good peroxidase, Jan. 5. {Strong peroxidase, Jan. 5.	{Jan. 5 {do.	{8 mosaic. {7 mosaic. Do.
Tap water (control).	.....	do.	Untreated.	do.	do.	do.
X <sup>2</sup>	July 12	Intense peroxidase.....	5 c. c. virus+15 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 13	{No peroxidase, July 17. {No peroxidase, Aug. 21.	{Aug. 21 {Aug. 21	{All healthy. {8 mosaic.
Do.	do.	do.	8 c. c. virus+12 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 13	{No peroxidase, July 17. {No peroxidase, Aug. 21.	{do. {do.	{Do. {Do.
Do.	do.	do.	10 c. c. virus+10 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 13	{No peroxidase, July 17. {No peroxidase, Aug. 21.	{do. {do.	{Do. {Do.
Do.	do.	do.	12 c. c. virus+8 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 13	{No peroxidase, July 17. {No peroxidase, Aug. 21.	{do. {do.	{1 mosaic. {4 mosaic.
Do.	do.	do.	15 c. c. virus+5 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 13	{Trace peroxidase, July 17. {No peroxidase, Aug. 21.	{do. {do.	{4 mosaic. {7 mosaic.
Do.	do.	do.	16 c. c. virus+4 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 13	{Slight peroxidase, July 17. {No peroxidase, Aug. 21.	{do. {do.	{7 mosaic. {4 mosaic.
Do.	do.	do.	18 c. c. virus+2 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 13	{Strong peroxidase, July 17. {No peroxidase, Aug. 21.	{do. {do.	{4 mosaic. {Do.
Tap water (control).	.....	do.	Untreated.	{Intense peroxidase, Aug. 21.	{do.	{All healthy.
X <sup>3</sup>	Mar. 28	Intense peroxidase, intense catalase.	5 c. c. virus+15 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 1	{No peroxidase, July 10. {No peroxidase, Aug. 23, but H <sub>2</sub> O <sub>2</sub> in excess.	{Aug. 23 {Aug. 23	{Do. {Do.
Do.	do.	do.	10 c. c. virus+10 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 1	{No peroxidase, July 6. {No peroxidase, Aug. 23.	{do. {do.	{10 mosaic. {7 mosaic.
Do.	do.	do.	15 c. c. virus+5 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 1	{No H <sub>2</sub> O <sub>2</sub> present on Aug. 23. {Strong peroxidase, July 10.	{do. {do.	{5 mosaic. {All healthy.
X <sup>4</sup>	July 8	Intense peroxidase, strong catalase.	10 c. c. virus+15 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 8	{Intense peroxidase, Aug. 23. {No peroxidase, but H <sub>2</sub> O <sub>2</sub> in excess Aug. 25.	{do. {Aug. 25	{Do. {Do.
Do.	do.	do.	10 c. c. virus+10 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 8	{No peroxidase, no H <sub>2</sub> O <sub>2</sub> . {No peroxidase, no H <sub>2</sub> O <sub>2</sub> .	{do. {Oct. 23	{9 mosaic. {4 mosaic.
Do.	do.	do.	15 c. c. virus+5 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 8	{No peroxidase, no H <sub>2</sub> O <sub>2</sub> . {No peroxidase, no H <sub>2</sub> O <sub>2</sub> .	{July 10. {Aug. 25	{7 mosaic. {7 mosaic.
Do.	do.	do.	17 c. c. virus+3 c. c. H <sub>2</sub> O <sub>2</sub> , prepared July 8	{No peroxidase, no H <sub>2</sub> O <sub>2</sub> . {No peroxidase, no H <sub>2</sub> O <sub>2</sub> .	{July 10. {Oct. 23	{8 mosaic. {4 mosaic.

Do.....	do.....	Untreated.....	{Intense peroxidase, Aug. 25. {Strong catalase, Aug. 25.....	Aug. 25 do.....	9 mosaic. All healthy.
Tap water (control).....	do.....	Untreated.....	{Intense peroxidase, Aug. 25. {Strong catalase, Aug. 25.....	Aug. 25 do.....	9 mosaic. All healthy.
X <sup>20</sup> .....	Nov. 1 Intense peroxidase, no catalase.	24 c. c. virus + 1 c. c. H <sub>2</sub> O <sub>2</sub> prepared Nov. 17.....	Good peroxidase, Nov. 18. Very weak peroxidase, Nov. 22. No peroxidase, Nov. 27.....	Dec. 21 do.....	7 mosaic.
Do.....	do.....	23 c. c. virus + 2 c. c. H <sub>2</sub> O <sub>2</sub> prepared Nov. 17.....	No H <sub>2</sub> O <sub>2</sub> , Dec. 20..... Weak peroxidase, Nov. 18. No peroxidase, Nov. 22.....	do.....	5 mosaic.
Do.....	do.....	22 c. c. virus + 3 c. c. H <sub>2</sub> O <sub>2</sub> prepared Nov. 17.....	H <sub>2</sub> O <sub>2</sub> present, Nov. 22..... No H <sub>2</sub> O <sub>2</sub> present, Dec. 20..... No peroxidase, Nov. 18.....	do.....	4 mosaic.
Do.....	do.....	21 c. c. virus + 4 c. c. H <sub>2</sub> O <sub>2</sub> prepared Nov. 17.....	No peroxidase, Dec. 26..... H <sub>2</sub> O <sub>2</sub> in excess, Dec. 26..... No peroxidase, Nov. 18.....	do.....	All healthy.
Do.....	do.....	20 c. c. virus + 5 c. c. H <sub>2</sub> O <sub>2</sub> prepared Nov. 17.....	H <sub>2</sub> O <sub>2</sub> in excess, Dec. 26..... No peroxidase, Nov. 18..... No peroxidase, Dec. 26.....	do.....	Do.
Do.....	do.....	19 c. c. virus + 6 c. c. H <sub>2</sub> O <sub>2</sub> prepared Nov. 17.....	Much excess H <sub>2</sub> O <sub>2</sub> , Dec. 26..... No peroxidase, Nov. 18..... No peroxidase, Dec. 26.....	do.....	Do.
Tap water (control).....	do.....	Untreated.....	Intense peroxidase.....	do.....	8 mosaic. All healthy.

## TREATMENT OF THE VIRUS WITH FORMALDEHYDE

Although formaldehyde destroys the infective principle of the virus in certain concentrations, peroxidase is not appreciably injured for a considerable time at much greater concentrations. Loew (15, p. 20) has shown that the peroxidase of tobacco is unaltered in a 5 per cent solution after 48 hours. It appears that some oxidases also are very resistant to formaldehyde. Kastle (13) found that the oxidase of the mushroom *Lepiota americana* is not destroyed by a 40 per cent formic-aldehyde solution which is allowed to act for several days. In the writer's test (Table VI), the peroxidase of tobacco was not appreciably changed in 1 per cent solutions of formaldehyde after standing 30 days. In these tests a 37 per cent U. S. P. solution of formaldehyde was used. All concentrations were made on the assumption that 2.5 c. c. of this solution contained about 1 gm. of formaldehyde. The virus was filtered through paper to remove all suspended material. To subject the virus to a certain strength of formaldehyde, a water solution of formaldehyde just twice as strong as desired for the virus was made up. Equal parts of this solution and the virus were then mixed, thus bringing the formaldehyde strength down to that required for the virus. In this way the virus was uniformly diluted to one-half its original strength in all concentrations of formaldehyde.

TABLE VI.—*Infectivity of the mosaic virus after 31 days' treatment with formaldehyde in 1915, 10 plants having been used in each test*

Virus used.	Enzymic reactions before treatment.	Strength of formaldehyde in the virus solutions.	Date prepared.	Enzymic reactions after treatment.	Date inoculated.	Result.
X <sup>20</sup> .....	Intense peroxidase.	1 : 100.....	Nov. 17	Intense peroxidase, Dec. 17.	Dec. 18	All healthy.
Do.....	do.....	1 : 200.....	do.....	do.....	do.....	Do.
Do.....	do.....	1 : 400.....	do.....	do.....	do.....	Do.
Do.....	do.....	1 : 600.....	do.....	do.....	do.....	Do.
Do.....	do.....	1 : 800.....	do.....	do.....	do.....	1 mosaic.
Do.....	do.....	1 : 1,000.....	do.....	do.....	do.....	Do.
Do.....	do.....	1 : 1,200.....	do.....	do.....	do.....	7 mosaic.
Do.....	do.....	1 : 1,500.....	do.....	do.....	do.....	5 mosaic.
Do.....	do.....	Untreated.	do.....	do.....	do.....	9 mosaic.
Tap water (control).					do.....	All healthy.

Experiments carried out in 1914 with unfiltered virus treated with the same strengths of formaldehyde and tested 32 days later gave practically the same results. In these tests the virus still retained its infectious properties in that solution which contained 1 part of formaldehyde in 1,000 parts of virus solution. All stronger solutions had lost the power to produce infection.

TREATMENT OF DRIED MOSAIC MATERIAL, WITH ETHER,  
CHLOROFORM, AND OTHER SOLVENTS

In the following experiments dried and ground mosaic material, designated as X<sup>13</sup>, was used. The original green leaves were harvested on August 31, 1915, and dried in the air. For each solvent the procedure was as follows: Ten gm. of air-dry material were extracted with 70 c. c. of extractive for two days. This solution was then filtered through paper and 35 c. c. of the filtered solution were set aside in a small beaker to evaporate at room temperatures. The residue left after evaporation was brought into 5 c. c. of distilled water and used for inoculation. The original residue, X<sup>13</sup>, left after filtering off the solvent, was then thoroughly dried at room temperatures and macerated with 50 c. c. of distilled water in a mortar. Ten c. c. of the extract were used for inoculation. In this way the infective properties of the extract and of the original material from which this extract was obtained could be compared.

The process was somewhat different with glycerin, as this is not readily evaporated. After extracting 10 gm. of the dry material for two days the glycerin extract was pressed out and filtered through hard paper under reduced atmospheric pressure. Of the filtered solution 40 c. c. were then made up to 800 c. c. with distilled water, giving a 5 per cent glycerin solution, which will produce no injury when inoculated into tobacco plants. After filtering off the glycerin extract the original X<sup>13</sup> residue was then subjected to pressure to remove as much glycerin as possible and was then macerated in a mortar with 50 c. c. of distilled water. The results obtained for each solvent are shown in Table VII.

TABLE VII.—Effect of digestion with ether, chloroform, and other solvents upon infectivity of mosaic material X<sup>13</sup> dried at room temperatures in 1915, 10 plants having been used in each test

Solvent used.	Period of extraction.	Time required for extract to evaporate.	Date taken up with water.	Peroxidase reaction after treatment.	Results of inoculating plants Oct. 19, 1915.
Residue X <sup>13</sup> after digestion with ether.	Oct. 6 to 8....	Oct. 8 to 16....	Oct. 16	Very weak..	3 mosaic.
Ether extract of X <sup>13</sup> .....	.....do.....	Oct. 8 to 12....	Oct. 12	None.....	All healthy.
Residue X <sup>13</sup> after digestion with chloroform.	.....do.....	Oct. 8 to 16....	Oct. 16	Very weak..	10 mosaic.
Chloroform extract of X <sup>13</sup> .....	.....do.....	Oct. 8 to 12....	Oct. 12	None.....	All healthy.
Residue X <sup>13</sup> after digestion with carbon tetrachlorid.	.....do.....	Oct. 8 to 16....	Oct. 16	Very weak..	5 mosaic.
Carbon tetrachlorid extract of X <sup>13</sup> .....	.....do.....	Oct. 8 to 12....	Oct. 12	None.....	All healthy.
Residue X <sup>13</sup> after digestion with toluene.	.....do.....	Oct. 8 to 16....	Oct. 16	Very weak..	10 mosaic.
Toluene extract of X <sup>13</sup> .....	.....do.....	Oct. 8 to 12....	Oct. 12	None.....	All healthy.
Residue X <sup>13</sup> after digestion with acetone.	.....do.....	Oct. 8 to 16....	Oct. 16	Very weak..	8 mosaic.
Acetone extract of X <sup>13</sup> .....	.....do.....	Oct. 8 to 12....	Oct. 12	None.....	All healthy.
Residue X <sup>13</sup> after digestion with ethyl alcohol.	Oct. 8 to 10....	Oct. 10 to 16....	Oct. 16	Weak.....	Do.
Ethyl alcohol extract of X <sup>13</sup> .....	.....do.....	Oct. 10 to 13....	Oct. 13	None.....	Do.
Residue X <sup>13</sup> after digestion with methyl alcohol.	.....do.....	Oct. 10 to 16....	Oct. 16	Very weak..	Do.
Methyl alcohol extract of X <sup>13</sup> .....	.....do.....	Oct. 10 to 15....	Oct. 15	None.....	Do.
Residue X <sup>13</sup> after digestion with glycerin.	.....do.....	Not evaporated.	Oct. 16	Very weak..	6 mosaic.
Glycerin extract of X <sup>13</sup> .....	.....do.....	.....do.....	Oct. 11	.....do.....	10 mosaic.
Residue X <sup>13</sup> after digestion with water.	.....do.....	Oct. 10 to 16....	Oct. 16	.....do.....	Do.
Water extract of X <sup>13</sup> .....	.....do.....	Not evaporated.	Oct. 11	Good.....	8 mosaic.
Tap water (control).....	.....do.....	.....do.....	.....do.....	.....do.....	All healthy.

In the test with glycerin the material to which the glycerin had been added was subjected to maceration and pressure in order to obtain the extract. A later experiment would seem to indicate that if the glycerin extract is poured off without subjecting the residue to maceration or pressure the extract will contain little, if any, of the infectious principle. In this experiment 10 gm. of the same X<sup>13</sup> material were used. This material, however, was dried over sulphuric acid in a desiccator from November 8 to November 23, 1915. On November 23, 80 c. c. of glycerin were added and allowed to stand until November 27, when the dark-colored extract was merely poured off and filtered through hard paper under reduced atmospheric pressure. Solutions containing 8 and 20 per cent of the extract were made, distilled water being used to dilute the glycerin.

The dry material from which the glycerin extract had been poured off was now washed with 100 c. c. of distilled water. This was poured off and filtered through hard paper, and the material was again washed with 380 c. c. of distilled water. This solution was also poured off and filtered through hard paper. The original leaf material, which was now fairly free from glycerin, was macerated with 25 c. c. of distilled water. The results of testing the above solutions and material for peroxidase and infection are given in Table VIII.

TABLE VIII.—Results of inoculations with dried material X<sup>13</sup> digested with glycerin in 1915, 10 plants having been used in each test

Material used.	Peroxidase reaction after treatment.	Date inoculated.	Result.
8 per cent glycerin extract.....	No peroxidase.....	{Nov. 27 Dec. 15} Dec. 16	1 mosaic.
20 per cent glycerin extract.....	do.....	do.....	All healthy.
100 c. c. water solution (first washing).....	do.....	do.....	1 mosaic.
380 c. c. water solution (second washing).....	do.....	do.....	4 mosaic.
Residue X <sup>13</sup> macerated with 25 c. c. H <sub>2</sub> O.....	Weak peroxidase.....	{Nov. 27 Dec. 15} do.....	10 mosaic.
Tap water only (control).....	.....	do.....	All healthy.

In order to compare the results with dried mosaic material, green mosaic leaf material was also treated with ether, chloroform, and water. For each solvent 25 gm. of finely cut and macerated green mosaic material were used. The quantity of solvent used was about 100 gm.—that is, 140 c. c. of ether, 80 c. c. of chloroform, and 100 c. c. of distilled water. These solvents were added to the green material and shaken on October 20. On October 21 all the solution that could be poured off was then filtered through hard paper and set aside in beakers to evaporate in the air. Of the filtered ether solution 115 c. c., and of the chloroform solution 55 c. c., were obtained. After evaporation, the residues left from the ether and chloroform solutions were each placed in 5 c. c. of distilled water. The leaf material from which these solutions had been obtained

was again dried at room temperatures and macerated with 20 c. c. of distilled water. The results of inoculation experiments with this material are shown in Table IX.

TABLE IX.—*Infectivity of green mosaic leaf material after digestion with ether, chloroform, and water, October 20 and 21, 1915, 10 plants having been used in each test*

Material used.	Time required for extract to evaporate.	Date taken up with water.	Peroxidase reaction after digestion.	Results of inoculating plants Oct. 25, 1915.
Green residue after digestion with ether.	Oct. 21 to 23.....	Oct. 23	Intense peroxidase, Oct. 25..	10 mosaic.
Ether solution from above...	Oct. 21 to 22.....	Oct. 22	Good peroxidase, Oct. 25....	6 mosaic.
Green residue after digestion with chloroform.	Oct. 21 to 23.....	Oct. 23	Intense peroxidase, Oct. 25..	10 mosaic.
Chloroform solution from above.	.....do.....	.....do.....	.....do.....	9 mosaic.
Green residue after digestion with water.	.....do.....	.....do.....	Good peroxidase, Oct. 25....	10 mosaic.
Water solution from above...	Not evaporated.....	.....	Intense peroxidase, Oct. 25..	Do.
Tap water (control).....	.....	.....	.....	All healthy.

From Table IX it is evident that the infective principle of the virus was not killed in the ether or chloroform solutions. From similar experiments Clinton (8, p. 415) believed that ether and chloroform could extract the virus from the green leaves to some extent without injury to its infectious properties.

However, from the fact that green crushed material contains a large amount of water, it is very probable that some of this water containing the infective principle passes into the ether or chloroform solutions. Such solutions would represent little more than mixtures of virus and ether, etc. Although the infective principle and likewise peroxidase appeared in the ether and chloroform solutions when green material was used, these did not appear in ether or chloroform extracts made with dry material.

The fact that the infective principle, or even enzymes, appeared in solutions obtained by adding ether, chloroform, toluene, etc., to green material does not justify the conclusion that such substances are soluble in these solvents. Kastle (13, p. 16), working with the oxidases of *Leptota americana*, found that if toluene is added to portions of the fresh fungus, some of the oxidase passes into the toluene layer. He says:

Whether the perfectly dry oxidase is soluble in toluene remains to be proved. It may be, of course, that it is the water which is dissolved in the toluene which really takes the oxidase into solution.

Various experiments have shown that the infective principle of the mosaic disease of tobacco is not readily destroyed by ether, chloroform, toluene, or carbon tetrachlorid. Although ether or chloroform vapors quickly kill the green leaf, the infective principle in a mosaic leaf killed in this way remains uninjured after several hours' treatment. Likewise

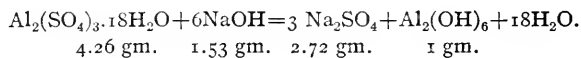
virus solutions to which several cubic centimeters of ether, chloroform, or toluene have been added did not lose their infectious properties after several months. Carbon tetrachlorid also appears to be quite as inert when added to virus solutions. In a test with this material, 3 c. c. were added to 22 c. c. of virus. The supernatant virus, when tested one month later, was quite as infectious as the untreated.

In other experiments the mosaic sap has been evaporated to dryness at room temperatures in beakers and the residue treated with ether for several days. Under such conditions, however, the residue is only slightly soluble in the ether and there remains a heavy, gummy, more or less impermeable mass. When the ether was evaporated and the residue again taken up with the original amount of water, the solution was still infectious.

Although Clinton (8, p. 415) states that the virus can be preserved for a long time by adding to it a small amount of toluene, the writer's experiments indicate that the virus will retain its infectious properties almost indefinitely without the addition of toluene. With no preservative whatever added, the bottled virus was highly infectious when tested from 12 to 15 months later, although putrefaction had taken place.

#### TREATMENT OF VIRUS WITH PRECIPITATES OF HYDROXIDS OF ALUMINUM AND NICKEL

Precipitation of the virus of the mosaic disease of tobacco by alcohol in 45 and 50 per cent strengths indicates that the precipitate carries down the infective principle, leaving the supernatant solution without infectious properties. Similar precipitation experiments have been carried out, using aluminum sulphate and nickel sulphate in alkaline solutions of virus to obtain the insoluble hydroxids of these metals. In order to obtain approximately 1 gm. of aluminum hydroxid in the precipitate aluminum sulphate and sodium hydroxid were added according to the following equation:



4.26 gm.    1.53 gm.    2.72 gm.    1 gm.

The procedure was as follows: On December 1, 1915, 100 c. c. of virus X<sup>23</sup>, which had been filtered through paper to obtain a clear solution, were made up to 1,000 c. c. with distilled water, thus diluting the virus but 10 times. First, 4.3 gm. of aluminum sulphate dissolved in a small quantity of water were added to the virus solution and shaken. Then 1.5 gm. of sodium hydroxid, dissolved in a small quantity of water, were added and the entire solution shaken and set aside. A very heavy flocculent precipitate of aluminum hydroxid was at once formed. This gradually settled, leaving the supernatant solution perfectly clear. On December 2 the solution was tested with litmus paper and gave a slightly



acid reaction. On this date the greater portion of the clear supernatant solution was carefully siphoned off. After pouring off as much of the remaining supernatant solution as possible, the semiliquid precipitate, or sludge, was bottled. The clear supernatant solution, as well as the sludge, gave intense peroxidase reactions.

As seen from the equation, the treatment of the original virus solution involves the formation of 2.7 gm. of the soluble salt (sodium sulphate) or 1 part in 370 of solution.

As a control to this there was prepared on December 1 a solution of virus of the same dilution as the original, containing 6 gm. of sodium sulphate per 1,000 c. c. of solution, or approximately 1 part of sodium sulphate in 303 parts of solution. It will be noted that this concentration is somewhat higher than that obtained in the reaction to produce 1 gm. of aluminum hydroxid.

The preparation of the nickel-sulphate solution, involving the formation of 2 gm. of nickel hydroxid, was carried out in the same manner as for the aluminum hydroxid. The results of inoculations made with the supernatant solutions and precipitates of aluminum hydroxid and nickel hydroxid are given in Table X.

TABLE X.—*Effect of aluminum hydroxid and nickel hydroxid upon the infectivity of mosaic virus, 10 plants having been used in each test. Material prepared on December 1, 1915*

Material tested.	Enzymic reaction after treatment.	Result of inoculating plants.
Inoculations made on December 16, 1915:		
Semifluid aluminum hydroxid precipitate.....	Intense peroxidase, Dec. 15, 1915.	1 mosaic.
Do.....	do.....	3 mosaic.
Supernatant solution from above precipitate.....	do.....	All healthy.
Semifluid nickel hydroxid precipitate.....	do.....	Do.
Supernatant solution from above precipitate.....	do.....	Do.
Sodium-sulphate-virus solution (1 part sodium sulphate in 303 parts of solution).....	do.....	10 mosaic.
Do.....	do.....	Do.
Original virus X <sup>23</sup> used in above tests, untreated.....	do.....	4 mosaic.
Tap water (control).....	do.....	All healthy.
Inoculations made with above material on January 18, 1916:		
Semifluid aluminum hydroxid precipitate.....	Intense peroxidase, Jan. 17, 1916.	6 mosaic.
Supernatant solution from above precipitate.....	do.....	All healthy.
Semifluid nickel hydroxid precipitate.....	do.....	1 mosaic.
Supernatant solution from above precipitate.....	do.....	All healthy.
Original virus X <sup>23</sup> used in above tests, untreated.....	do.....	10 mosaic.
Tap water (control).....	do.....	All healthy.

From the results of Table X it is quite evident that the infective principle of the virus was carried down with the aluminum hydroxid precipitate, leaving the supernatant solution free from infectious properties. Since the treatment with nickel sulphate appears to have destroyed the virus entirely, it is possible that nickel salts are more toxic to the infective principle than the salts of aluminum.

## EFFECT OF HEAT UPON THE VIRUS OF THE MOSAIC DISEASE

Several investigators have noted the effect of heat upon the virus of the mosaic disease. Mayer (17, p. 451) found that continued heating at 60° C. did not perceptibly change the infectivity of the virus, but that temperatures of 65° to 75° weakened it. Its infectious properties were completely destroyed when the virus was heated for several hours at 80°.

TABLE XI.—*Effect of heat upon the infectivity of undiluted solutions of mosaic virus heated without previous filtering, in 1915, 10 plants having been used in each test*

Virus used.	Enzymic reaction before treatment.	Date and nature of treatment.	Enzymic reaction after treatment.	Date inoculated.	Results.
X <sup>11</sup> ....	Intense peroxidase	Heated 15 minutes at 82° C. in test tube suspended in beaker of water, Nov. 23.	Good peroxidase, Nov. 24.	Nov. 24	10 mosaic.
Do.....	do.....	Boiled 1 minute in test tube, Nov. 23.	do.....	do.....	All healthy.
Do.....	do.....	Boiled 5 minutes in test tube, Nov. 23.	Weak peroxidase, Nov. 24.	do.....	Do.
Do.....	do.....	Boiled 10 minutes in test tube, Nov. 23.	do.....	do.....	Do.
Do.....	do.....	Unheated.....	Intense peroxidase, Nov. 24.	do.....	8 mosaic.
X <sup>9</sup> .....	do.....	Heated 10 minutes at 85° C. in test tube suspended in beaker of water, Dec. 2.	Fair peroxidase, Dec. 3.	Dec. 3	9 mosaic.
Do.....	do.....	Heated 15 minutes at 85° C. Nov. 23; again heated 7 minutes at 88° C., Dec. 3.	No peroxidase, Dec. 4.	Dec. 4	All healthy.
Do.....	do.....	Heated 10 minutes at 90° C. in test tube suspended in beaker of water, Dec. 2.	Weak peroxidase, Dec. 3.	Dec. 3	Do.
Do.....	do.....	Heated 10 minutes at 95° C. in test tube suspended in beaker of water, Dec. 2.	Very weak peroxidase, Dec. 3.	do.....	Do.
Do.....	do.....	Heated 1 to 2 minutes at 100° C. in test tube suspended in beaker of water, Dec. 2.	Fair peroxidase, Dec. 3.	do.....	Do.
Do.....	do.....	Unheated.....	Intense peroxidase, Dec. 3.	Dec. 4	8 mosaic.
Control	Strong peroxidase.	Tap water and healthy juice, untreated.	Strong peroxidase, Dec. 4.	do.....	All healthy.
X <sup>65</sup> .....	Intense peroxidase	Heated 5 minutes at 85° C. in test tube suspended in beaker of water, May 8.	Good peroxidase, May 8.	May 10	9 mosaic.
Do.....	do.....	Heated 5 minutes at 87° C. in test tube suspended in beaker of water, May 8.	do.....	do.....	8 mosaic.
Do.....	do.....	Heated 5 minutes at 88° C. in test tube suspended in beaker of water, May 8.	do.....	do.....	Do.
Do.....	do.....	Heated 5 minutes at 90° C. in test tube suspended in beaker of water, May 8.	Fair peroxidase, May 8.	do.....	1 mosaic.
Do.....	do.....	Heated 5 minutes at 91° C. in test tube suspended in beaker of water, May 8.	do.....	do.....	All healthy.
Do.....	do.....	Heated 5 minutes at 93° C. in test tube suspended in beaker of water, May 8.	do.....	do.....	Do.
Do.....	do.....	Heated 5 minutes at 95° C. in test tube suspended in beaker of water, May 8.	Very faint peroxidase, May 8.	do.....	Do.
Do.....	do.....	Unheated.....	Intense peroxidase, May 8.	do.....	7 mosaic.
Control	Strong peroxidase.	Tap water and healthy juice, untreated.	Strong peroxidase, May 10.	do.....	All healthy.

Iwanowski (12) and, likewise, Beijerinck (4) found that heating the virus to the boiling point destroyed its infectious properties.

According to Koning (14, p. 71-86), who heated the diluted virus in closed tubes, it remained infective when heated 10 minutes at 80°, 5 minutes at 90°, and 5 minutes at 100°.

Woods (20, p. 17-19) believed that the sap of mosaic plants remained infectious to some extent after it had been boiled, owing to the fact that peroxidase was regenerated in the solution.

The writer's experiments indicate that the infective principle of the virus is quickly and permanently destroyed at temperatures near the boiling point, although such solutions may again show good peroxidase reactions. In these tests, in which the test tube containing the virus was suspended in a beaker of heated water, the virus was kept at room temperatures until immersed. The test tube was immersed when the temperature in the beaker had begun to exceed the required point. Owing to the small quantities of virus used, the temperatures in all instances were very quickly brought up to the desired height.

The infective principle of the disease withstands much higher temperatures when the dried mosaic leaf material is subjected to dry heat. In the following experiments the air-dried mosaic leaves were finely ground and dried over sulphuric acid in a desiccator from October 8 to the date of heating. For each test 5 gm. of this powdered material were heated in an electric oven, then macerated and extracted with 25 c. c. of distilled water. The results shown in Table XII were obtained.

TABLE XII.—*Effect of heat upon the infectivity of dried mosaic leaf material in 1915, 10 plants having been used in each test*

Date heated.	Period of heating.	Enzymic reaction after treatment.	Date inoculated.	Results.
Oct. 15	½ hour at 90° C.	Fair peroxidase, Oct. 22.	Oct. 23	10 mosaic.
Do...	½ hour at 100° C.	Trace peroxidase, Oct. 22.	do...	Do.
Do...	One hour at 100° C.	do.	do...	7 mosaic.
Do...	½ hour at 110° C.	do.	do...	9 mosaic.
Do...	½ hour at 120° C.	No peroxidase, Oct. 22.	do...	7 mosaic.
	Dry material, unheated.	Fair peroxidase, Oct. 22.	do...	10 mosaic.
	Tap water only, unheated.		do...	All healthy.
Nov. 5	½ hour at 140° C.	No peroxidase, Nov. 10.	Nov. 12	Do.
Do...	½ hour at 150° C.	do.	do...	Do.

Heating experiments were again carried out, using the virus after it had been evaporated to dryness in small beakers. For each test 40 c. c. of undiluted and unfiltered virus N<sup>16</sup> were allowed to evaporate by exposure to the air on October 10, 1915. On November 4 the beakers containing the air-dried residues were heated in an electric oven. After being heated the residues were immediately taken up with 30 c. c. of distilled water.

TABLE XIII.—Effect of heat upon the infectivity of mosaic virus which has been evaporated to dryness at room temperatures in 1915, 10 plants having been used in each test

Date heated.	Period of heating.	Enzymic reaction after treatment.	Date inoculated.	Results.
Nov. 4	½ hour at 100° C.....	{Fair peroxidase, Nov. 11..... {Trace catalase, Nov. 11.....	Nov. 12	All healthy.
Do...	½ hour at 110° C.....	{No peroxidase, Nov. 11..... {No catalase, Nov. 11.....		
Do...	½ hour at 120° C.....	.....do.....	..do....	Do.
Do...	½ hour at 130° C.....	.....do.....	..do....	Do.
	Original virus evaporated air-dry, but not heated.	{Weak peroxidase, Nov. 11..... {Intense catalase, Nov. 11.....	..do....	5 mosaic.

From the data in Table XIII it is evident that the evaporated virus solution lost its infectious properties much more quickly than the dried and ground mosaic leaf material. Likewise, the peroxidase was somewhat more quickly destroyed in the evaporated material. Although the presence of small amounts of moisture in the air-dried residue of the evaporated virus may have hastened the destruction of the infective principle in this material, it is also possible that the infective principle is better able to withstand high temperatures when allowed to remain within the tissues of the leaf.

Although the virus with which Koning (14, p. 71-86) worked appears to have withstood temperatures as high as 100° C., the virus with which the writer worked is very quickly destroyed at temperatures above 90°. In some instances, however, the virus has been rendered noninfectious at a temperature 10 degrees lower than this. These results were obtained with the virus designated as XI<sup>16</sup>. This virus was extracted from tobacco plants on September 20, 1915, and bottled until used on October 18, 1915. Although the unheated virus was highly infectious, the infective principle was destroyed after heating for 5 minutes at 80°. It was also destroyed when heated for 2 minutes at 81°. Although the original, unheated virus gave intense reactions for catalase and peroxidase, the catalase was completely destroyed in these tests. Weak peroxidase reactions, however, were again shown the next day.

As Woods (20) has shown, there is frequently a return of peroxidase activity in solutions of virus that have been once heated. This activity does not appear to return immediately after cooling, but usually requires some hours for its return. Woods considered that the enzyme was destroyed in such solutions but a resistant zymogen again generated more of the peroxidase after cooling. On the other hand, Hasselbring and Alsberg (9) were led to believe from their experiments that a zymogen might not be present, but that the enzyme was included and protected in the coagulum and subsequently leached out on standing.

By heating the virus several times at 85° C., the writer has been able in some instances to destroy completely the peroxidase present without destroying the infective principle. The highly infectious virus designated as X° and showing intense peroxidase reactions was treated as

follows: Heated 10 minutes at 85° on December 2, 1914. Cooled at once. A fair peroxidase reaction shown on December 3. Again heated 10 minutes at 85° on December 4, and cooled at once. Weak peroxidase reaction shown December 5. Again heated 10 minutes at 85° on December 5 and immediately cooled. Very weak peroxidase reaction was shown on December 16, when it was again heated for the fourth time for 15 minutes at 85°. When used for inoculation on December 28, no peroxidase reaction was shown. The virus was still highly infectious, however, and produced the mosaic disease in 9 out of 10 plants.

EFFECT OF LOW TEMPERATURES UPON THE VIRUS

After having been frozen for periods varying from one to four hours at -12° C., the extracted sap of mosaic plants still retained its infectious properties unchanged. It likewise retained its original virulence after having been exposed outdoors during the entire winter of 1915 and allowed to freeze and thaw repeatedly. In recent experiments liquid air was used to freeze the virus, and a temperature of approximately -180° was reached. The results are given in Table XIV.

TABLE XIV.—*Effect upon infectivity of freezing fresh mosaic sap to -180° C. by means of liquid air in 1916, 10 plants having been used in each test*

Material used.	Time exposed to liquid air.	Peroxidase reaction before freezing.	Peroxidase reaction after freezing.	Result of inoculating plants, Feb. 2, 1916.
	<i>Minutes.</i>			
Original virus, unfrozen.....	.....	Intense, Feb. 1.....		10 mosaic.
Original virus, frozen Jan. 31.....	15	.....do.....	Intense, Feb. 1.....	Do.
Duplicate of above.....	15	.....do.....	.....do.....	Do.
Tap water and healthy sap, unfrozen.....	.....	.....do.....	.....do.....	All healthy.

These tests indicate that the infective principle of the mosaic disease of tobacco is highly resistant to extremely low temperatures.

DISAPPEARANCE OF PEROXIDASE IN MOSAIC VIRUS WITHOUT LOSS OF INFECTIOUS PROPERTIES

It has been observed in several instances that unpreserved solutions of virus, as well as dried and ground mosaic material, may lose their peroxidase activities and still retain infectious properties. This happened with dried and ground mosaic leaves bottled in December, 1912. This material showed fair peroxidase reactions on January 28, 1915, but no reactions for peroxidase in October, 1915. At this time the virus still retained the power to produce infection.

In another instance a bottle of unpreserved virus which was extracted on April 27, 1914, failed to give peroxidase reactions on December 3, 1914; yet at this time was highly infectious, producing the disease in 9 plants out of 10 inoculated. This virus was also highly infectious when tested on May 15, 1915, producing the mosaic disease in 8 out of 10 plants. Although the virus was not tested for peroxidase at the time it was extracted, the fresh virus would probably have shown peroxidase

reactions. In the writer's experience, freshly extracted sap from healthy plants as well as from plants affected with the mosaic disease has never failed to give more or less intense peroxidase reactions.

Various experiments have shown that talc-treated virus slowly loses its peroxidase activities, although still retaining its infectious properties, as shown in the following test: On November 19, 1915, 50 c. c. of virus X<sup>20</sup>, extracted on November 1, 1915, and filtered through paper, were mixed to a thick paste with 72 gm. of powdered talc, U. S. P. This material was tested for peroxidase reaction and infectivity from time to time with the following results:

Strong peroxidase reaction.....	Nov. 19, 1915.
Very weak peroxidase reaction.....	Nov. 30, 1915.
Very faint peroxidase reaction.....	Dec. 20, 1915.... 10 plants mosaic.
No peroxidase reaction.....	Jan. 18, 1916.... 4 plants mosaic.

Similar results have been noted when the virus, and also green mosaic material, have been buried in the soil. The virus, and likewise the green material, may entirely lose their peroxidase activities on decaying, although still retaining the power to produce infection.

#### INFECTIOUS PROPERTIES LOST AND PEROXIDASE ACTIVITIES RETAINED

While the infective principle of the mosaic disease appears to be very resistant, the infectivity of a virus solution may be lost under some conditions, although the peroxidase is not appreciably changed. This was noted as a result of evaporating a quantity of virus to dryness. A solution of 350 c. c. of virus which had been extracted some time previously and allowed to undergo free fermentation was evaporated at room temperature from September 18 to October 21, 1915. On October 21 the solution had been reduced to 20 c. c. of a thick, heavy, putrid-smelling black sirup. Although this solution gave much more intense reactions for catalase and peroxidase than the original solution, showing that these enzymes had been concentrated during the process of evaporation, the infective principle of the virus had been completely destroyed. Inoculation tests showed that the original virus, however, still retained its infectious properties.

Although in this instance the infective principle had been destroyed, many tests have shown that the virus of the mosaic disease is not usually destroyed, even when evaporated to dryness.

Experiments with the feces of hornworms fed upon the leaves of mosaic plants have given rather interesting results. After the worms had been feeding upon the plants for a day or two the feces were collected and macerated with distilled water. In one test the feces of a single worm were used. Out of 10 plants inoculated, one plant only became diseased. Since but one case of the mosaic disease appeared in this test, there is a possibility that this plant developed the disease as a result of accidental infection from other sources.

In another experiment six hornworms which had been feeding upon mosaic plants in the field were transferred to mosaic plants in the laboratory and left for a day or two. The feces were then collected, macerated with tap water, and tested as follows: Ten plants were inoculated with the extracted sap of mosaic leaves upon which the worms were allowed to feed. Nine plants became mosaic. Ten plants were inoculated with a water extract of the feces of the hornworms. All remained healthy. Ten plants were inoculated with tap water (control). All remained healthy.

Although these results indicate that the infective principle of the original material had been destroyed by the digestive process of the worms, the feces gave intense peroxidase reactions.

INFECTIVE PRINCIPLE OF THE DISEASE NOT A NORMAL  
CONSTITUENT OF THE SAP OF HEALTHY PLANTS

Woods, from his cutting-back experiments with tobacco and other plants, was led to believe that the mosaic disease of tobacco had its origin within the cells of the plants as a result of abnormal physiological activities. Although Woods ascribed the origin of the disease to peroxidase, he believed that there was no essential difference between the peroxidase of healthy and that of diseased plants and came to the conclusion that this enzym obtained from either source could produce the disease.

In an earlier paper (1) the writer has adduced evidence to show that the disease is not produced by simply cutting back or otherwise subjecting plants to unfavorable conditions. In the present paper it has also been shown that peroxidase bears no essential relation to infection and that by various methods this and other enzymes may be more or less completely removed from the virus without affecting the infective principle of the disease, and vice versa.

Although the sap of healthy plants may be rich in oxidase, peroxidase, and catalase, such sap never produces the mosaic disease in healthy plants. Although the peroxidase of diseased plants may be decreased to such an extent by dilution with distilled water that it can not be detected by the guaiac-hydrogen-peroxid test, the solution still remains highly infectious. The results of the experiments in which the virus was diluted with distilled water make this plain (Table XV).

TABLE XV.—*Effect of dilution of mosaic virus with distilled water*

Degree of dilution.	Peroxidase reaction.	Number of plants inoculated.	Result.
Virus undiluted.....	Intense.....		
1 part virus in 250 parts water.....	Weak.....		
1 part virus in 500 parts water.....	None.....	10	8 mosaic.
1 part virus in 1,000 parts water.....	do.....	10	6 mosaic.
Tap water only (control).....		10	All healthy.

On the other hand, by evaporation the enzymes present in the sap of healthy plants may be brought to the highest possible concentration, but such solutions never acquire infectious properties.

That oxidase (producing the blue color with guaiac alone) can not be responsible for the mosaic disease may be shown by heating the solution to 70° C. for several minutes. This temperature destroys the oxidase, according to Loew (15; p. 31), but does not affect the peroxidase or the principle of infection. As a matter of fact, the oxidase of the tobacco sap appears to be an unstable enzyme and very soon disappears entirely from untreated solutions on standing.

Although the enzyme termed "catalase" by Loew is very often a normal constituent of healthy and mosaic plants, it can be shown that the presence of this enzyme has nothing to do with infection. As shown by Loew (16, p. 19), catalase is destroyed by heating the solution for a minute or two at 80° C. Such solutions, although no longer showing reactions for catalase, may yet retain their infectious properties.

Although it is known that other enzymes than oxidase, peroxidase, and catalase occur normally in the sap of healthy tobacco plants (18), such enzymes can not be considered in a causal relationship to the mosaic disease if it has been established that this disease is not of so-called physiological or spontaneous origin—that is, it can not occur in the absence of infection. Furthermore, the writer sees no reason to believe that any specific enzyme occurs in a mosaic tobacco plant which would not be found in healthy plants.

Although it has been shown by various workers that the enzymic relations and reactions in plants become disturbed as a result of disease and unfavorable conditions of growth, there is no reason to believe that these disturbances, when associated with the mosaic disease of tobacco, hold a causal relation to the disease. It is now well known that various factors, aside from pathological conditions caused by an unknown infective principle, may change the quantitative relations of enzymes in plants, as Bunzel (5) has shown in studying the curly-top of sugar beets. It yet remains to be shown that an increase in the amount or activity of enzymes in diseased plants is anything more than a symptom or an indication of disturbed metabolism as a result of the disease.

In the writer's experience all evidence at hand indicates that the mosaic disease of tobacco is dependent upon a specific pathogenic agent which must be introduced into healthy plants from without before the disease can arise. That this pathogenic entity is highly infectious and is in some manner reproduced within the plant are established facts. If these facts are interpreted according to those fundamental principles upon which all our scientific conceptions in pathology and biology are based, that infectious diseases are associated with parasitism and that self-reproduction is a characteristic of living things alone, it must be admitted that the pathogenic agents responsible for the mosaic disease of tobacco must be parasites. If from the facts stated above it is held that nonliving chemical substances such as enzymes or toxins engender the disease, our fundamental biological conceptions no longer hold true.



## SUMMARY

In this paper are given the results of a study of the properties of the virus of the mosaic disease of tobacco, and evidence is adduced to show that the infective principle can not be identified with peroxidase. Briefly, the facts obtained may be stated as follows:

(1) The infective principle of the mosaic disease of tobacco is retained by the Livingstone atmometer porous cup used as a filter, and also by powdered talc. Although the filtrates may show intense peroxidase reactions, they no longer produce infection.

(2) The infective principle of the disease is quickly destroyed in alcohol of a strength of 75 to 80 per cent. In this strength precipitation of the peroxidase is complete. By filtering the solution the peroxidase may be collected, freed from alcohol by evaporation, and redissolved with water. This solution gives intense peroxidase reactions, but no longer produces infection. Alcoholic solutions of virus of 45 and 50 per cent strengths did not destroy the infective principle of the disease within the same period. In these solutions the pathogenic agents are not destroyed and appear to be carried down with the precipitate, leaving the supernatant solution without infectious properties, although giving strong peroxidase reactions.

(3) By the addition of different quantities of hydrogen peroxid to the virus, it is possible to find a concentration of sufficient strength to destroy the peroxidase, but leaving little or no free peroxid in the solution. Such solutions no longer show peroxidase reactions, but retain their infectious properties for a long time. A considerable excess of hydrogen peroxid destroys the infective principle itself. The quantity of hydrogen peroxid required to destroy the peroxidase without leaving any considerable excess in the solution depends upon the nature of the virus, the amount of active catalase present, etc.

(4) The virus was treated with formaldehyde for 31 days in the following concentrations: One part formaldehyde in 100, 200, 400, 600, 800, 1,000, 1,200, and 1,500 parts of virus solution. The solutions containing 1 part formaldehyde in 800, 1,000, 1,200, and 1,500 parts of solution gave infection. Stronger concentrations were no longer infectious, although giving intense reactions for peroxidase.

(5) Ether, chloroform, carbon tetrachlorid, toluene, and acetone failed to extract either the infective principle or the peroxidase from dried mosaic material. These solvents also failed to destroy the infectious principle in this material. Ethyl and methyl alcohol completely destroyed the infective principle in the leaf material itself, as well as in the extract. No evidence of peroxidase was obtained in the alcohol extracts. Glycerin does not destroy the infective principle of the disease. Water extracts of dried material not only show peroxidase reactions, but also contain the infective principle of the disease.

(6) A precipitate of aluminum hydroxid, formed by adding aluminum sulphate to alkaline solutions of virus, appeared to carry down the infec-

tive principle of the disease, leaving the clear, supernatant solution without infectious properties, although showing good peroxidase reactions. Similar treatment with nickel sulphate was not so satisfactory, as it gave evidence of being more toxic to the infective principle than the aluminum salt.

(7) The virus is quickly killed at temperatures near the boiling point of water. In some instances heating the virus for five minutes at 80° C. was sufficient to destroy its infectivity. In other tests, with a different virus solution, heating for five minutes at 90° did not entirely destroy its infectivity. In dried and ground mosaic material, rendered water-free by drying over sulphuric acid in a desiccator, the infective principle resisted much higher temperatures than it did in solutions. If virus solutions are heated, the thermal death point of the infective principle is lower than that of the peroxidase; or at least it is more quickly destroyed than the peroxidase.

(8) The virus is highly resistant to low temperatures. When frozen to a temperature of -180° C. with liquid air, its infectious properties were not weakened.

(9) Unpreserved solutions of virus have sometimes lost their peroxidase activities without losing their infectious properties. Dried and ground mosaic material has also lost its peroxidase activities and still remained highly infectious. Talc-treated material, while retaining its infectious properties, has lost its peroxidase activities.

(10) Solutions of virus sometimes lose their infectious properties and continue to show intense peroxidase reactions, as when allowed to evaporate spontaneously in one instance. The feces of worms fed upon mosaic plants have, in some instances, failed to produce infection, although such material continued to give intense peroxidase reactions.

(11) The writer's experiments show that peroxidase or catalase in the sap of mosaic plants can not be responsible for the mosaic disease. The same enzymes are normally present in healthy plants, but the sap of such plants is without infectious properties. By evaporation the enzymes present in healthy sap may be brought to a high concentration, and such solutions never acquire infectious properties. By dilution, on the other hand, the peroxidase content of mosaic sap may be diminished to such an extent that peroxidase reactions are no longer discernible; yet such solutions may remain highly infectious.

Since it has been shown that the mosaic disease of tobacco does not occur in the absence of infection, neither enzymes nor other normal constituents in the sap of healthy plants can be considered responsible for the disease. A specific, particulate substance not a normal constituent of healthy plants is the cause of the disease. Since this pathogenic agent is highly infectious and is capable of increasing indefinitely within susceptible plants, there is every reason to believe that it is an ultramicroscopic parasite of some kind.

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## PLATE XCI

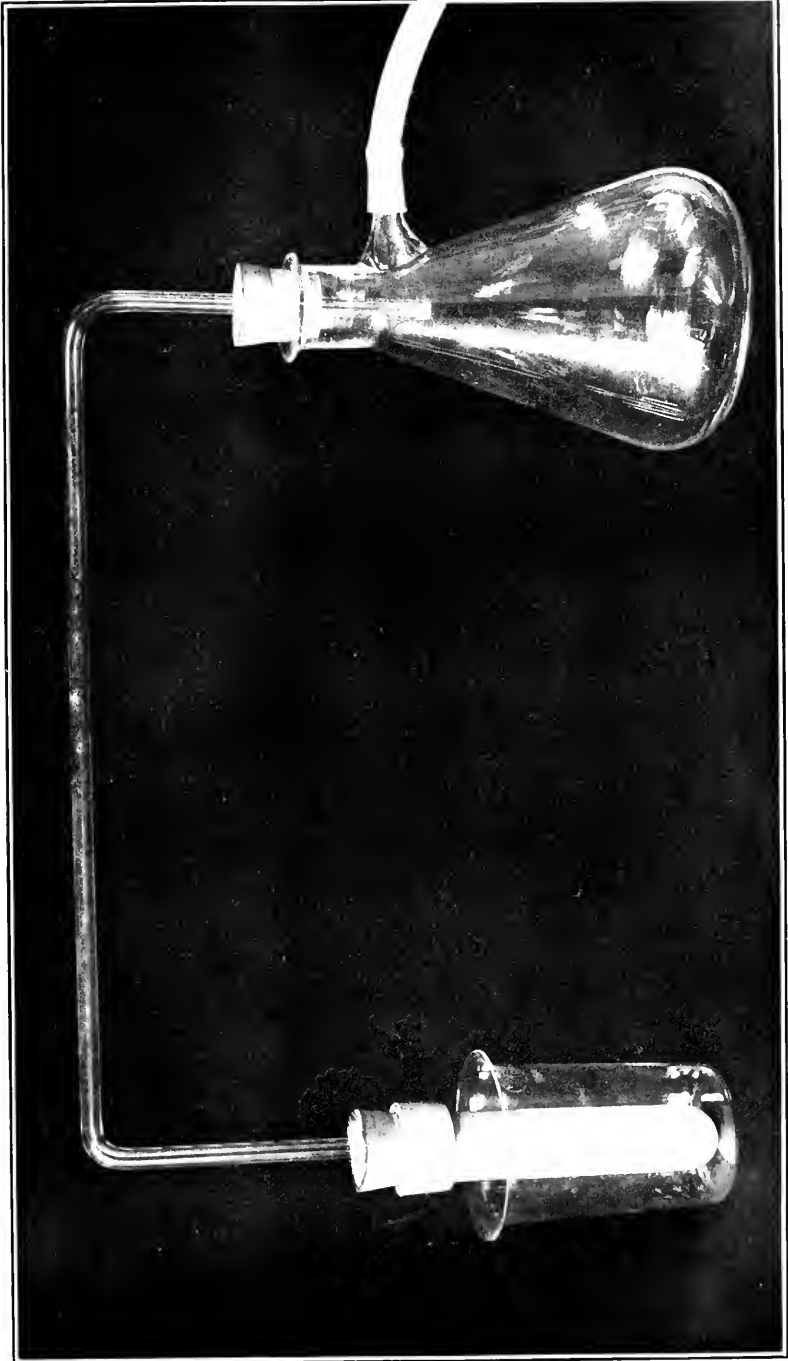
Livingstone atmometer porus cup as used for filtration. The virus of the mosaic disease of tobacco always lost its infectious properties in passing through this filter.

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## LIFE CYCLES OF THE BACTERIA

[PRELIMINARY COMMUNICATION]

By F. LÖHNIS, *Soil Biologist*, and N. R. SMITH, *Scientific Assistant, Bureau of Plant Industry*.<sup>1</sup>

### INTRODUCTION

Two years ago the senior author, together with J. Hanzawa (14),<sup>2</sup> published the results of some *Azotobacter* studies, showing for the first time that the large *Azotobacter* cells are a special type of growth of a spore-forming bacillus. We said (14, p. 2):

Es steht jetzt fest, dass in der Tat die grossen, sporenfreien *Azotobacter*-Zellen Wuchsformen eines schlanken, Endosporen bildenden *Bacillus* sind. *Bacillus Azotobacter* ist demnach die korrekte Bezeichnung für diese Art.<sup>3</sup>

As we had to discontinue our investigations at that time, we pointed out (14, p. 6) that further research in this direction would be very desirable:

Sicherlich würden weitere Forschungen in dieser Richtung noch manchen für den Systematiker wie für den Physiologen gleich wichtigen Einblick erschliessen.

In the meantime some new papers on *Azotobacter* have been published. But they merely confirmed once more certain facts concerning the normal growth and the rather general occurrence of *Azotobacter* in soils. Only one author, Mulvania (15), reports the presence of heat-resisting spores. In the other cases no spores were observed. However, they undoubtedly would have been found by a more thorough search. One of these authors readily admitted this fact in a letter, saying that his statement had been made "on the basis of the ordinary examination always made by soil bacteriologists."

<sup>1</sup> The photomicrographs, as well as the final drawing of the text figure accompanying this paper, have been made by Mr. F. L. Goll, of the Bureau of Plant Industry. Grateful acknowledgment is due him for his very careful work.

<sup>2</sup> Reference is made by number to "Literature cited," p. 701-702.

<sup>3</sup> In accordance with the usage of K. B. Lehmann and most of the other European bacteriologists, we apply the name "*Bacillus*" to the spore-forming rods. As *Bacillus azotobacter*, like most, probably all, spore-forming rods, has, at least temporarily, peritrichous flagella, its name would also be valid if Migula's system should be preferred. However, we fully agree with Lehmann, that this system is especially unsatisfactory.

As our earlier observations indicated that an extended study would lead to still more interesting results, we have resumed our work. A comparative study of 24 *Azotobacter* cultures and 18 strains of other bacteria now revealed the fact that those wide morphological differences first observed with *Azotobacter* are by no means restricted to this one group of bacteria. Similar variations occurred with all cultures tested, and under suitable conditions they will occur with all bacteria generally. The importance of these wide morphological variations, however, is materially increased by the fact that they are connected with no less considerable variations in the physiological qualities of those organisms. Therefore, not only for diagnostic and systematic purposes are these facts of fundamental importance but also for all other lines of research in agricultural and medical bacteriology.

The quite unexpected character of the results obtained seems to justify a preliminary discussion of the facts and problems involved. Of course, at the present time it is neither our intention to furnish all those numerous details which are necessary to obtain a full knowledge of these heretofore practically unknown facts, nor do we want to collect all the widely scattered observations from a voluminous literature which will not only give some interesting support to our new viewpoint but which also, in their turn, will sometimes find their full explanation there. At present we merely wish to inform agricultural and also medical bacteriologists about these newly discovered facts and to ask for their cooperation.

It is beyond question that progress in bacteriology has been severely checked by the widespread inclination to consider as not worth studying or as some uninteresting "involution form" all that sort of bacterial growth which does not fit exactly into the conventional conception of a very simple and constant character of the species. Even modern standard works assert, for instance, that the branched type of *Bacillus radicum* represents an "involution form" not capable of further propagation. However, nitrogen fixation takes place only when these branched forms develop, which unmistakably proves their full virility; and there is no lack of exact results which show conclusively that suitable conditions always allow a new development from these branched forms.

Undoubtedly a somewhat more scientific study of such "abnormal" forms would long ago have revealed the fact that the life cycles of the bacteria are no less complicated than those of many other micro-organisms. Indeed, numerous items in the bacteriological literature, for instance, show that the formation of gonidia and the budding of bacteria have been observed quite frequently. Yet again the authoritative statement that bacteria multiply exclusively by fission apparently has been sufficient to prevent thorough research in this direction, and the credulous adherence to "standard methods" unfortunately explains only too well why the turning point in the life cycles of the bacteria has been com-

pletely overlooked. In fact, this slime or granulated dirt has been merely an annoying occurrence on the slides of thousands of bacteriologists. Acetic acid and many other remedies have been recommended to insure clean preparates. Of course, beef broth and some other substrates usually give really dirty smears which need some cleaning, but we have been much too radical in this direction. Under certain conditions all bacteria pass over into a "symplastic" stage, appearing under the microscope as either an unstainable or a readily stainable mass without any visible organization, which, if not discarded as dead, later gives birth to new regenerated forms frequently of very characteristic and unusual appearance.

As practically all our new knowledge of the life cycles of the bacteria has been derived from a renewed study of *B. azotobacter*, the behavior of this organism will be described first.

Before we enter into this subject, however, we beg to point out that by discussing the life cycles of the bacteria we do not intend to revive any of those unclear theories concerning bacterial polymorphism or pleomorphism. The development of the bacteria is characterized not by the *irregular* occurrence of more or less *abnormal* forms but by the *regular* occurrence of many different forms and stages of growth connected with each other by *constant relations*.

Unquestionably many so-called species frequently described in the most superficial manner will have to be canceled, because they merely represent fragments of the life cycles of other bacteria. "Good" species, on the other hand, will not only keep their position, but they will receive a much more complete and sharper definition than they now have. Moreover, the discovery of the symplastic stage opens the way to answer by exact experiments the question concerning species or varieties.

#### THE LIFE CYCLE OF BACILLUS AZOTOBACTER

In text figure 1 is given a schematic sketch of the development of *B. azotobacter* according to our present knowledge. The letters A to M indicate the different types of growths which are separated from each other by broken lines. The single- and double-pointed arrows show the connections between the different forms as they have actually been observed. Each of the four circles contain in each case all those forms which have heretofore been considered by careful investigators as representing sufficient basis for establishing a species. Observers of the more usual, less painstaking class, however, have been only too much inclined to form new species even inside these subcycles. For example, the different types of spore-free and spore-bearing rods, all included in our type F, could easily have induced authors like Migula and Matzushita to create half a dozen "species" of that sort; perhaps this really happened.

With the exception of D and H, all these types have been observed and described in earlier publications on *Azotobacter* and closely related

spore-forming bacilli (*B. malabarensis*, *B. danicus*, and *B. oxalaticus*). However, figure 1 shows clearly how just this type D, which has hereto-

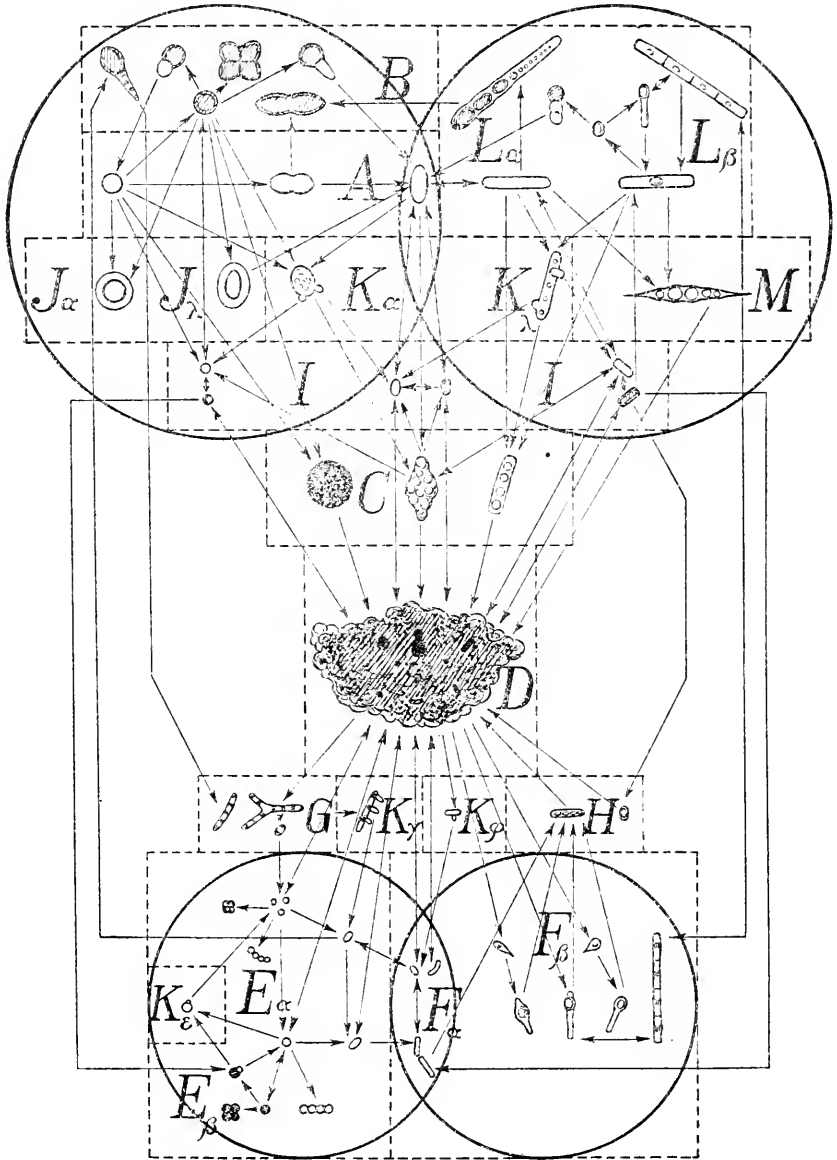


FIG. 1.—Life cycle of *Bacillus azotobacter*. The broken straight lines divide the different types of growth indicated by the letters A to M. The Greek letters  $\alpha$  to  $\lambda$  refer to subdivisions. The single- and double-pointed arrows indicate the development of one form from another. The four circles confine, in every case, all those forms which represent together a rather constant mode of life and which have been usually considered as bases for establishing separate species.

fore so successfully dodged all bacteriologists, represents practically the key to a correct understanding of the whole problem. There are some

direct outside connections between the larger and the smaller forms, as indicated by the six arrows: I-E, E-B, B-G, and I-F, F-L, I-H; but they are rather rare exceptions. The symplastic stage D, on the other hand, can be observed in all cultures. Even if a strain shows little or no inclination to change from one subcycle into another, it regularly passes through type D. By frequent successive transfers (made each morning and evening), we tried to prevent this "breakdown." For some days we were successful, but then this disintegration again took place. After another couple of days the tendency to produce "normal" forms was once more very pronounced, which in its turn was again supplanted by the formation of type D. We have followed this rhythmic alternation for some weeks with *B. azotobacter* as well as with *B. subtilis*.

It goes without saying that the arrangement and naming of the different types is merely a matter of convenience. Several of them perhaps could be split up into two or more. However, we found them so suitable in their present form for our work that we do not see any necessity for making an alteration.

Type A represents the normal, well-known, large *Azotobacter* cells of globular or oval form (usually 2 to 3 $\mu$  broad and 3 to 5 $\mu$  long). By further stretching they pass over into type L (fig. 1 and 2 of Pl. A).

Type B indicates the thick-walled, rather resistant "arthrospores," regularly formed from type A.<sup>1</sup> When they germinate, either globular or oval forms are liberated. Some cells of type B, however, are produced occasionally by D, or still less frequently by a direct enlargement of small cells of type E or F, which will be discussed later. In the latter case the germ developing from B shows the character of type G. Probably no B formed by A germinates in this manner. This fact, then, would distinguish these morphologically identical forms.

Type C comprises all large forms in the stage of granular decomposition heretofore generally considered to be dying "involution" forms. If the granules are very small, they are nearly always easily stained. The larger ones, on the other hand, usually remain entirely unstained when treated with aqueous solutions of anilin dyes. Owing to the degenerated condition of the cell wall, the form of the cells frequently becomes quite irregular, and the granular content may become partially or entirely free (fig. 7 to 10 of Pl. B). Undoubtedly these granules are of different nature. Some may be fat, glycogen, or other metabolic products. Most of them however, are living entities, as is clearly shown by their further behavior, if not by their motility. Sometimes these granules develop to full-sized cells before being liberated. (See type J.) In this case they behave exactly like the "gonidia" in iron bacteria, as described by Cohn (4). However, in most cases they either leave the cell entirely before they

<sup>1</sup> We think it best to reserve the name "arthrospore" exclusively for those cases where the whole cell acquires the character of a spore. If only parts of the cell (either at its end or side) show such transformation, we call them "regenerative bodies" or "exospores," according to their special character.

start growing, or they grow out of it, piercing the cell wall. With all small bacteria we have observed only the two latter types of growth. Almquist (1), who made some similar observations, called these granules "conidia." In our opinion the term "gonidia"—that is, seed—is preferable, as these granules in every case act as organs of propagation and multiplication, whatever may be their special mode of growth.

Type D is in most cases the dissolution product either of the large forms (types C and M) or of the small cells (types E, F, and H), but it can also be formed by typical spores of type L, by regenerative bodies (I) and by gonidia. Its inclination to take the stain varies widely. If the cell walls participate considerably in its formation, it is readily and deeply stained. The same holds true when the gonidia are small and easily stainable. The large unstainable gonidia, on the other hand, which are frequently produced in type C, as well as in type H (see below), naturally give a rather pale or entirely unstained D. The structure, too, varies accordingly. Small cells, or small gonidia, cause a finely granulated, somewhat "hairy" structure; especially in the case of small slender rods like *B. fluorescens*, *B. radiobacter*, etc., the term "woolly" perhaps would be applicable. Large gonidia, on the other hand, as well as spores, clearly melt together when entering this stage of growth. Figures 7 to 12 (Pl. B), 18 (Pl. C), and 19 (Pl. D) illustrate the different possibilities. Like type C, type D has been considered by some investigators—for example, by K. B. Lehmann—as an occurrence indicating the death of the bacteria.<sup>1</sup> Usually, however, it has been passed as some uninteresting "slime" or "dirt." As it is made up by a thorough mixing or melting of a frequently large number of cells, spores, or gonidia, the term *sympiasm* or *symplastic stage* seems to be a correct and convenient name for this stage.

Some time after the sympiasm has been formed, very small granules, *regenerative units* (0.2 to 0.3 $\mu$ ), become visible. If the sympiasm does not take the stain, the appearance of these organized well-stained forms inside the amorphous pale mass is very surprising (fig. 12 of Pl. B). Such a preparate indeed first turned our attention into this new direction. The regenerative units increase in size until they show the form of type E, F, I, or even A or B (fig. 13 and 14 of Pl. C). All these cells are easily stained, their cell walls being usually comparatively thick. At last, practically all the sympiasm is reorganized, leaving sometimes only very few pale small "flakes."

Type E represents a miniature counterpart of types A and B. The size of the cells varies between 0.3 and 1 $\mu$ . Only with the latter forms are the thin and the thick walled cells clearly discernible. In some cases at least, we were able to observe germinating arthrospores of this type. If necessary, both subtypes may be indicated conveniently by appending to the E a Greek letter  $\alpha$  or  $\beta$ , respectively.

<sup>1</sup> The absurd name "zoogloea," which means "animal slime," has been repeatedly applied to this product of bacterial "autolysis," and the fact that the walls of the cells are dissolved has been considered as indicating the death of the content of the cells.

Type F comprises all small rodlike cells of different form, straight or curved, about 0.3 to 0.5 $\mu$  broad, 0.75 to 1.5 $\mu$ , or more, long. When not forming spores, they may be labeled F $\alpha$ ; otherwise F $\beta$ . Cells of the F $\alpha$  type occasionally look very much like *B. radiobacter* and related species. In cases where great difficulties were encountered in getting a pure culture of *B. azotobacter*, this type of growth probably has repeatedly displayed an unwelcome activity. When developing from the symplastic stage, type F $\beta$  shows different and somewhat unusual-looking intermediate forms (fig. 15 of Pl. C). For making a spore-bearing rod of the "Plectridium" type, a body splits off from the symplasm, showing a comparatively large "head" and a very small pointed "tail." When the tendency prevails, however, to form a "clostridium", the well-stained regenerative unit is located inside a pale sheath with pointed ends and in growing stretches until the albuminous substance is equally distributed inside the cell wall. Later, a part of the protoplasm once more concentrates, developing the spore. Spore-free, as well as spore-bearing, thin threads can directly, without passing through type D, change into the large type L. (See below.) On the other hand, they can also originate directly from this type (fig. 20 of Pl. D). In the latter case they sometimes resemble type G, from which they differ, however, by their spore formation and their genesis.

Type G shows, when treated with aqueous fuchsin, unevenly stained, frequently branched threads looking very much like Actinomyces. It is, however, as indicated in text figure 1 and shown in figure 16 (Pl. C), merely an intermediate step between types D and E which may be dispensed with. The small cells of type E are kept together by unstainable slime. Boiling water dissolves this slime within two minutes. A prepate treated accordingly with boiling aqueous fuchsin shows merely type E or some threads just dissolving (fig. 17 of Pl. C).

Type H acts as the counterpart of type C. There the larger, here the smaller cells are undergoing a granular decomposition leading to type D. However, rods, as well as spores, show a very unusual appearance in this case. They become entirely unstainable by aqueous dyes, but remain clearly visible even with a wide-open condenser, owing to the very bright luster of their granular content (fig. 18 of Pl. C).

Type I represents the globular, oval, or rodlike "regenerative" bodies which have been studied by Prazmowski (18). Here, again, an added  $\alpha$  or  $\beta$ , respectively, may indicate their more or less resistant character (thin or thick cell wall). I $\alpha$  usually originates from types A and B (fig. 5 of Pl. A) or from type C (fig. 7 of Pl. B). I $\beta$ , on the other hand, in most cases is an offspring from the symplastic stage D (fig. 13 of Pl. C). Irregularly shaped type I, which is quite frequent with the other bacteria, has been observed only occasionally in cultures of *Azotobacter* (fig. 16 of Pl. C). The regenerative bodies either produce, by germinating or by stretching, cells of type A, B, or L or they convert themselves entirely into

regular spores. This possibility will be discussed at once. But, as mentioned before, regenerative bodies may also produce forms belonging to types E and F, making two of the "outside" connections between the upper and the lower circles as given in text figure 1.

Type J characterizes another rather rare occurrence also studied by Prazmowski. Forms belonging to type A, B, or occasionally L, increase in size and inside themselves develop one or more new full-sized cells of type A or B (fig. 6 of Pl. A and fig. 21 of Pl. D). These new cells are the result of the growth of the gonidia.

Type K comprises all those cells of type A, B, E, F, G, I, or L which produce one or more well-stained, round, oval, or rodlike buds, which, in the case of the large forms, occasionally cause a close resemblance to some budding yeast cell. These "buds" are gonidia developing into regenerative bodies, seldom directly into full-sized forms. An added  $\alpha$ ,  $\beta$ ,  $\epsilon$ ,  $\varphi$ ,  $\gamma$ ,  $\iota$ , or  $\lambda$  indicates the relation to type A, B, E, F, G, I, or L, respectively (fig. 3 to 6 of Pl. A; fig. 14, 16, 17 of Pl. C; fig. 20, 21, 24 of Pl. D).

Type L is made up of the large spore-free and spore-forming rods  $L\alpha$  and  $L\beta$ , as well as of free spores and long threads. Germinating spores of this type produce either long rods or rather short ovals resembling type A. The big spore-free rods and threads resulting from type A (fig. 1, 2 of Pl. A) seem to be unable to develop directly the faculty to form endospores. At least, we have never observed such a change, and this also would be in accordance with the fact that a direct transformation of a spore-free into a spore-forming bacterium has never been observed. As mentioned above, type  $F\beta$ , too, does not develop from type  $F\alpha$ , but directly from the symplasm. As is also noted above, these small spore-forming rods occasionally convert themselves into large spore-forming bacilli. Usually, however, the regenerative bodies formed by type D seem to be the normal predecessors. Under conditions, which will have to be studied more closely, these round cells acquire the tendency to produce endospores, which, in their turn, go back into the symplastic stage. This second symplasm then produces another set of regenerative bodies which stretch out to large granulated rods and threads. They later form the normal endospores.

Type M represents another rather unusual form. It originates from type L and passes over into type D (fig. 10 of Pl. B and fig. 19 of Pl. D).

It is hardly necessary to point out that sometimes our separation of the forms observed into different types becomes more or less arbitrary. For example, there are no absolutely sharp lines separating types A and B or the regenerative bodies I from the full-grown cells. Thick-walled cells of types E and F, when produced in the symplasm, might just as well be considered as "regenerative bodies." A germinating cell of type  $E\beta$  and a budding form of the type  $K\epsilon$  resemble each other very





More complete studies, of course, will fill all places now vacant in the table. The rather irregularly observed types G, H, J, and M are of no fundamental importance in the life cycle of *B. azotobacter*, and our interest has not been especially centered upon them. The large forms (types A, B, and L), as well as the small ones (types E and F), have been found in all cases. The same holds true concerning types D and I. This means that the full life cycle typical for *B. azotobacter* has been observed with every strain. That budding forms (type K) were also noticed in 23 out of 24 cases is of minor interest, because this is another type which represents no essential link in the cycle of bacterial life. Merely its unusual and heretofore nearly unknown appearance naturally attracted our attention.

The growth of the gonidia which causes this "budding" occasionally leads to three other kinds of development which deserve some short discussion. If the "buds" stretch out considerably, they cause the formation of branched bacteria, which can be found in the *Azotobacter* group, as well as in all other groups of bacteria. If, however, several gonidia, inclosed in the same cell or in its liberated granular content, start growing simultaneously in different directions, starlike forms result, which are frequently found in cultures of rodlike bacteria. *Bact. radiobacter* probably is the best known example of this special type of growth. But we have already reserved for a later publication good pictures of starlike outgrowths of slender rods from the typical large globular *Azotobacter* forms. An interesting crownlike form, representing the medium stage between simple budding and the formation of stars, is shown in figure 16 (Pl. C) directly above the upper symplasm. The third kind of gonidial development is another heretofore unknown type of growth. The budding gonidia sometimes develop into forms which clearly show the morphological and physiological character of typical endospores. Since they are produced, however, outside of the cell, they may be called *exospores*. In figure 20 (Pl. D) different stages of their development are reproduced. Their occurrence is not quite so surprising as it may seem to be at first, when considered in the light of the two following facts. Those "granules" which precede the formation of the normal endospores inside the bacterial cells are actually nothing else than small gonidia. When budding, the gonidia frequently develop into thick-walled regenerative bodies, which not only germinate in the same manner as endospores but may also acquire quite a considerable resistance against heat. As mentioned above, regenerative bodies growing out of the symplasm sometimes convert themselves practically entirely into spores. Budding *exospores* therefore are merely a special application of a general rule; they are regenerative bodies with the character of spores.

Normal heat-resistant endospores showing polar germination have been found in 13 of our *Azotobacter* cultures—that is, in more than 50

per cent of all cases. During our first investigations upon this subject (14) only 4 of 11 strains (No. 2, 4, 5, and 6) possessed this faculty, which they had acquired between 1908 and 1912. Culture 7 gave some bodies looking like endospores in 1914, but they were not resistant to heat. This faculty has now been fully developed. Cultures 15, 17, and 19, also typical spore-free cells at the time of their isolation some years ago, showed their inclination to form endospores when first tested in this direction in 1915. Cultures 23 to 25 developed this special character during the time we were experimenting with them. To fix exactly the conditions for transforming a spore-free into a spore-bearing strain will be one of the tasks in our later, detailed investigations. As already indicated, a close study of the symplasm and of the regenerative bodies derived from it will solve this problem as well as many others concerning the multitude of forms inclosed in the life cycles of the bacteria.

Unquestionably in all such experiments the inner condition of the cells is of no little importance. That at least in some directions this factor can eventually outmatch the influence of the outer conditions to a considerable extent is clearly shown by the interesting behavior of 22 of our *Azotobacter* strains when they were inoculated into soil extract containing 1 per cent of mannite and 0.05 per cent of monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ), after having grown previously in moist sterilized soil and mannite. Fifteen of these cultures grew in the large types A and L, and seven in the small types E and F when the experiment started. Two weeks later, without a single exception, all the fifteen strains changed from types A, B, and L to D, E, and F. Vice versa, the seven others produced types D and I, these developing into types A, B, and L. This result corroborates once more our statement that every strain of *Azotobacter* or of any other bacillus will pass through all phases of its cycle of life persistently if the conditions are suitable. Undoubtedly and in full accordance with the behavior of higher organisms, some strains are especially inclined to grow mostly in one or the other subcycle. However, the formation of the symplasm and its "plasticity" enables us, if we make use of these interesting possibilities, to induce and accelerate changes in the general development of a bacillus to which the special strain perhaps may be only very little inclined at that time. For example, our five newly isolated *Azotobacter* cultures 21 to 25 had, of course, the pronounced tendency to grow in their typical large globular or oval form. Only in about 1-month-old mannite solutions were the long rodlike forms more numerous, mixed with forms belonging to types A, B, D, E, F, and I. Transfers on mannite agar, after one day, gave in four cases an abundant and practically pure growth of the large rods, showing a tendency to form endospores. Only one culture (No. 24) failed, because in this case we had no such old solution at hand and had started, therefore, from a 3-day-old culture, which exclusively produced round and oval regenerative bodies on mannite agar.

Mannite soil extract, which has been used with very satisfactory results by the senior author (13) for more than 10 years for the study of nitrogen-fixing and other soil organisms, unquestionably has the disadvantage of a varying and partially unknown chemical composition. However, beef broth and similar substrates are liable to the same objection and yet are generally used in bacteriological laboratories. Nevertheless it would be preferable to have media at our disposition of the same favorable qualities but of well-defined chemical composition. Practically all of the many artificial substrates recommended in the bacteriological literature are much too concentrated, especially for soil organisms. For many of our experiments we used the following mineral solution to good advantage:

Monopotassium phosphate neutralized to phenolphthalein	
by sodium hydroxid.....	0.02 per cent
Magnesium sulphate.....	.02 per cent
Sodium chlorid.....	.02 per cent
Calcium sulphate.....	.01 per cent
Ferric chlorid, 1 per cent solution.....	2 drops per 100 c. c.

As carbonaceous material for *B. azotobacter*, 1 per cent mannite was used. The further addition of 0.02 per cent of potassium nitrate or peptone proved to be beneficial though not necessary. All these solutions are entirely clear and therefore especially suitable for microscopical studies. If a strip of filter paper sufficiently long to reach about 1 inch out of the solution is placed into the test tube before sterilizing, a luxuriant growth of the large forms belonging to types A and B quickly spreads on the part above the liquid.<sup>1</sup> In old solutions the symplasm frequently develops to such a degree that it becomes clearly visible to the naked eye as white flakes or slimy threads. Figure 14 (Pl. C) is a reproduction of the end of such an enormous accumulation of living material.

The last three pictures of our *Azotobacter* series (fig. 22 to 24 of Pl. D) illustrate one of the comparatively rare direct connections between the small and the large forms. We have here before us the exact counterpart of the alteration shown in figure 20 (Pl. D). Certainly this direct growing up of the small organisms to forms belonging to type B, the forthcoming of threads of type G in the germinating process, and the unusual appearance of their budding, by which the small forms are regenerated, deserve our full attention. However, this kind of development seems to be a rather rare exception to the rule. These forms also are much inclined to turn into the symplastic stage. A photographic picture of this occurrence will be published later.

The close study of this side connection, however, led us to another discovery which we had failed to make before, although our other prepares,

<sup>1</sup> The arrangement mentioned above is very helpful for obtaining pure cultures of *Azotobacter* from the soil. At the same time it allows the motility of an organism to be determined macroscopically. One of our strains crept up 20 cm. in 10 days on long paper strips in large test tubes.

as reproduced in figures 1 to 3 (Pl. A) and 8 (Pl. B), show the same fact much more clearly. In all these preparates many cells are in a conjunct stage, which can not be explained by the assumption that this *conjunction*<sup>1</sup> is only accidental. Most of these illustrations have been made from contact preparates taken directly from 4-day-old colonies. Smears made in accordance with the "standard methods" probably would have destroyed most of these connections. But also in this case it was still more the effect of our theoretical blinders which prevented an earlier seeing and understanding of this fact, which, like the budding of the bacteria and the formation of the symplasm, has not only actually been seen by many bacteriologists but also has been unknowingly reproduced in several illustrations in our daily used textbooks.

So far as we are aware, only one author has spoken of a similar observation. In 1892 Förster (6) found occasionally that *Chromatium Okenii* sometimes entered into some "primitive copulation." Among the drawings accompanying his paper, a sketch made from a photomicrograph seems to us most trustworthy. Its conformity with our *Azotobacter* illustrations is practically complete. Observations in the hanging-drop clearly showed that there is some interference between the plasmatic substances in the conjunct cells or even some direct mixing of them.

The determination of the actual physiological significance of this conjunction must be left, of course, to a more thorough investigation. At present we merely wish to add and to emphasize that this process is by no means such an exception as might be deduced from Förster's statements and from the silence observed in this direction in our textbooks. The conjunct stage seems to be of no less general importance and occurrence in bacterial life than the formation of the symplasm. Not only normal cells and regenerative bodies but also exospores have been frequently found in conjunction. And if we only succeed in forgetting for a moment our most cherished theories and simply try to look at the facts as they are, we find at once that the formation of the symplasm and the conjunction of the cells are nothing else than two modes of mixing plasmatic substances temporarily inclosed in separate cells and that evidently the continuity and rejuvenescence of the living matter in the bacteria is just as much dependent on this process as in the case of all other organisms.

A thorough study of the relations existing between the conjunct and symplastic stage will be the first object of our further investigations in this line. We hope that experiments with well-defined varieties and species will soon furnish a correct insight. The ease with which the "flakes" of the symplasm can be isolated is, of course, very advantageous for these, as well as for systematic, studies.

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<sup>1</sup> We prefer the new term *conjunction* instead of "copulation" or "conjugation," because frequently more than two cells unite and no sexual differentiation so far has been observed.

Before entering a discussion of the life cycles of other bacteria, the serial numbers for the four subcycles of *B. azotobacter* may be given, determined, so far as possible, in accordance with the methods recommended by the Society of American Bacteriologists. The behavior in the presence of the different carbonaceous substances, however, had to be tested in our mineral solution with nitrate to which 0.5 per cent of the different sugars, etc., was added, the highly concentrated peptone solution not being suitable for this organism. That the appearance of the colonies, as well as the other cultural characteristics, differs accordingly, goes without saying; these details will also be given later. The serial numbers resulting from our tests are as follows:

Type A.—221.2322813.

Type E.—222.2222524.

Type L.—121.3332033.

Type F.—122.4442034.

#### THE LIFE CYCLES OF OTHER BACTERIA

The following 18 cultures were selected as representatives of practically all groups of bacteria.

- No. 31. *B. subtilis*, isolated from evaporated milk.
- No. 32. *B. lactis niger*, Gorini's original culture from Kral's Museum.
- No. 33. *Tyrothrix tenuis*, Duclaux's original culture from Kral's Museum.
- No. 34. *B. danicus*, isolated from soil.
- No. 35. *Bact. pneumoniae*, isolated from soil.
- No. 36. *Bact. radiobacter*, isolated from soil.
- No. 38. *Bact. denitrificans agile*, Ampola's original culture from Kral's Museum.
- No. 39. *Bact. radicola*, isolated from vetch.
- No. 40. *Bact. fluorescens*, isolated from milk.
- No. 41. A yellow bacillus (not determined) isolated from soil.
- No. 42. *Planosarcina ureae*, Beijerinck's original culture from Kral's Museum.
- No. 43. *Sarcina flava*, isolated from milk.
- No. 44. *Micrococcus candidans*, isolated from chernozem.
- No. 45. *Micrococcus candidans*, isolated from evaporated milk.
- No. 46. Salt-water spirillum isolated from Great Salt Lake, Utah.
- No. 47. Salt-water spirillum isolated from sea water.
- No. 48. *Streptococcus lactis*, kindly furnished by Dr. L. A. Rogers, Bureau of Animal Industry.
- No. 49. *Bact. bulgaricum*, kindly furnished by Dr. L. A. Rogers.

Before being tested, these cultures had been grown on the following substrates:

- Beef agar: No. 31-36, 38, 40, 41, 43-45.
- Beef agar plus 3 per cent of sodium chlorid: No. 46, 47.
- Beef agar plus 0.5 per cent of urea: No. 42.
- Saccharose agar: No. 39.
- Milk: No. 48, 49.

After having been examined as to their purity on agar plates, they were cultivated on the different agars and in suitable solutions. Beef, salt, and urea agars were used as before. In the case of *B. radicola* (No. 39),

however, the saccharose agar was substituted by mannite agar as prepared for *B. azotobacter*. The milk organisms (No. 48 and 49) were transplanted on yeast-whey agar and into yeast-whey solution, these media being the most suitable for these organisms, according to the earlier observations of the senior author (13). The salt-water spirilla were grown in beef broth with 3 per cent of sodium chlorid. For the other organisms various solutions were prepared by adding to the mineral solution as used for *B. azotobacter* the following ingredients:

- 0.1 per cent of ammonium citrate plus 0.3 per cent of glycerin for cultures 31, 33 to 36, 38, 40, 41, 44, and 45.
- 0.04 per cent of peptone plus 0.3 per cent of glycerin for cultures 32, 42, and 43.
- 0.02 per cent of potassium nitrate plus 1 per cent of mannite for culture 39.
- 0.04 per cent of peptone plus 0.5 per cent of lactose for cultures 48 and 49.

All cultures were kept at 28° C. with the only exception of those of *Bact. bulgaricum* (No. 49), for which a temperature of 40° to 45° C. is more suitable.

In the light of the results obtained by us with *B. azotobacter* it was not difficult to find out that the life cycles of all these organisms are essentially the same. On all good substrates they all pass quite regularly through most, if not all, of the types of growth first observed with *B. azotobacter*. In Table II the results are summarized. Type N, which we have added here, represents the starlike growth previously mentioned.

TABLE II.—Types of growth observed with 18 representative bacteria

[The laboratory numbers of the cultures are given at the head of the columns]

Types of growth.	<i>B. subtilis</i> .	<i>B. lactis niger</i> .	<i>Tyrodhric tonnis</i> .	<i>B. danicus</i> .	<i>Bact. putumontiae</i> .	<i>Bact. radiobacter</i> .	<i>Bact. dentrificans aqlic.</i>	<i>Bact. raditricola</i> .	<i>Bact. fluorescens</i> .	Yellow bacillus.	<i>Flonocarcina ureac.</i>	<i>Sarcina flavu.</i>	<i>Micrococcus canalicans</i> (soil).	<i>Micrococcus canalicans</i> (milk).	Salt Lake spirillum	Ocean spirillum.	<i>Streptococcus lactis</i> .	<i>Bact. bulgaricum</i> .
	31	32	33	34	35	36	38	39	40	41	42	43	44	45	46	47	48	49
A (large globules and ovals).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B (thick-walled forms of type A).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C (granular decomposition of A, B, L, M).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D (sympiasm).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
E (small globules and ovals).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F (small rods and threads).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
G (slime threads with cocci).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H (granular decomposition of F and spores).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I (regenerative bodies).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
J (normal cells developing inside).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
K (budding gonidia).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L (large rods and threads).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
M (cells with pointed ends).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
N (starlike growths).....	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The disintegration of the normal cells into the sympastic stage (D), the formation as well as the further development of regenerative bodies

(I), and the occurrence of gonidia budding out of the normal cells (K) have been observed in every case. With all those cultures which regularly produce endospores (No. 31-34, 41, 42), large cells belonging to types A, B, C, and L, were observed; and they were not observed with the same constancy in cultures 35-40, 43-48. Of all these organisms none are known to produce endospores. *Bact. bulgaricum* (No. 49), too, is spore-free; but closely related forms isolated from the stomach have been reported as producing endospores. The formation of cells of type B, C, and L, makes it highly probable that an experimental trial to induce spore formation may soon be successful. However, the same possibility is by no means excluded in the other cases. It may be that small spore-forming forms can be branched off from the other cultures. Indeed, we have already obtained some quite encouraging results in this line. Whether, then, another progression to the large cell types will be possible is entirely an open question.

Referring again to our introductory remarks, we take this opportunity to point out specifically that these perhaps somewhat surprising statements should by no means be considered merely as some absurd polymorphistic hypothesis. The well-known character of *Bact. pneumoniae*, for example, will by itself remain completely unchanged, whatever may be the result of further investigations upon the full life cycle of this organism. If there is a spore-forming type, and perhaps even genetic relations with some large-sized cells, this would in no way interfere with nor impair the well-established facts already collected. Such wide morphological differences must always be connected with no less considerable alterations of the whole physiological character, so that these other types, if they are known, of course, are stored away as entirely different "species" in various remote places in the so-called "system" of bacteria. This conclusion can be drawn with absolute certainty from our observations on *B. azotobacter* as well as from Henri's experiments (10) with *B. anthracis*. If only those changed forms, frequently seen in all bacteriological laboratories, had not been persistently discarded as uninteresting "involution forms" or as "contaminations," the whole situation would undoubtedly be much clearer and much more satisfactory. At present it is not our intention to dwell upon the numerous details collected in our studies of the life cycles of the different organisms. Though the broad types of growth are the same with all, the morphological details, of course, differ considerably. Figures 25 to 30 (Pl. E), 31 to 36 (Pl. F), and 37 to 40 (Pl. G) will furnish sufficient proof in this direction, especially when compared with our illustrations of *B. azotobacter*. It may suffice to add the following remarks:

Figures 25 to 27 (Pl. E) illustrate the appearance of the same cultures of *B. subtilis* on a beef-agar slant, made from a 1-day-old colony on a beef-agar plate. The smear made directly from the colony showed



the typical spore-forming rods as reproduced in the atlas of Lehmann and Neumann (11, pl. 47, fig. V). Figure 25 (Pl. E) was made from a preparate from a 2-day-old agar slant. Spores dissolve into stage D; and thick-walled, more or less irregular regenerative bodies are being formed. This process is going on in the 6-day-old culture (fig. 26 of Pl. E). The "melting" of the spores is clearly visible. The regenerative bodies have increased in number as well as in size. Some forms resemble very much those of *B. radiccicola*. After eight days (fig. 27 of Pl. E) these regenerative bodies are either dissolved entirely into a readily stainable symplasm or they produce bright granulated spores (type H), which later also pass over into the symplastic stage. Sometimes the unstainable content of the regenerative bodies slips out of the dark-stained cell wall, forming an unstainable symplasm like that frequently produced by cells of the C type of *B. azotobacter* (fig. 6 of Pl. A; fig. 7 of Pl. B. See also the mixture of stained and unstained symplasm in fig. 19, Pl. D). The new set of regenerative bodies developing from the symplasm, especially from the dark-stained material derived from those irregular forms, usually showed rodlike forms stretching out into long granulated threads, which, in their turn, divided themselves into the normal spore-forming rods typical of *B. subtilis*.

This behavior was observed, only slightly modified, with all cultures of spore-forming rods. Figure 28 (Pl. E) shows this regeneration of the new threads from the symplastic stage as it was found in a 2-day-old transfer of the "yellow bacillus" (No. 41), made from a 12-day-old peptone-glycerin solution into the same liquid substrate. The thread on the right side of the field illustrates the situation especially well. As the upper part is broken off, the gonidia inside the cell, which caused the formation of the short branch on the lower part, become visible. The symplasm still contains several regenerative units which apparently are checked by the vigorous absorptive action of the thread.

The special appearance of many types of growth of *Bact. bulgaricum* is plainly discernible in figure 29 (Pl. E), made from a 6-day-old stab culture in yeast-whey agar. Large and small rods (types L and F), pointed forms (type M), the formation of regenerative bodies (type I) budding (type K) are clearly visible. On the left side of the figure two long, thin threads grow ("branch" or "bud") out of the same point in a larger rod. Below this another thick rod, showing granular decomposition (type C), is reproduced. In the middle of the field some thin, pale symplasm (D) is spread out. Above, a thin pale thread containing darker stained bodies (type G) crosses the field. Some small cells of type E are lying close to it. That the round cells budding out of the rods are indeed regenerative bodies is proved by their germination, the new rods growing out in one or in two directions. This frequently happens when the regenerative bodies are still connected with their mother cell.

The formation of the symplasm in an 11-day-old ammonium-citrate-glycerin solution of *B. fluorescens* is shown in figure 30 (Pl. E). This figure should be compared with figure 11 (Pl. B), showing the formation of stained symplasm of *B. azotobacter*. The dark rods also visible in the former figure are of the H type.

In figure 31 (Pl. F) cells of *Sarcina flava* from a 1-day-old beef-agar slant are reproduced partially disintegrating into the symplastic stage. The small symplasm in the center has already entered the formation of regenerative units. Many of the cells are in the conjunct stage.

Figure 32 (Pl. F) illustrates the transformation of the symplasm of *Streptococcus lactis* into many normal forms and some round regenerative bodies, as observed in a 5-day-old peptone-lactose solution. As far as this transformation has already taken place, it is clearly discernible that indeed, as mentioned before, the whole material is used again for the reproduction of new cells practically without leaving any remnants. Figure 33 (Pl. F) shows another "flake" of symplasm of *Streptococcus lactis* from a 3-day-old milk culture containing many regenerative units and some globular regenerative bodies. This illustration is of special interest for the following reasons: Such globular bodies of different diameters are produced by all kinds of bacteria (cf. fig. 13 of Pl. C; fig. 25, 26, 27 of Pl. E). If they are dispersed in their symplasm and this is embedded in the equally deeply stained casein of the milk, it looks nearly as if the albuminous substances of the milk were forming granules which later produce normal bacteria by germinating or stretching. The center of figure 33 (Pl. F), where a rather compact symplasm is lying above a very thin film of casein, shows that these things are entirely separate and different. However, in the lower left part of the field some symplasm is embedded in a thicker layer of casein, and here the situation is much less clear. Now, Fokker (5), one of the few authors who are still fighting in favor of spontaneous generation, has repeatedly pointed out that his standpoint is strongly supported by the fact that the albuminous substances in animal tissues, as well as in milk and in blood, produce small granules which later develop into normal bacteria. The assumption that his substrates were not sterile, of course, does not furnish a complete explanation of these peculiar observations. We believe, however, that our discovery of the symplasm and of its regenerative units settles this question.

That the formation of the symplasm and the regeneration of new cells are by no means an abnormal occurrence merely caused by the unnatural conditions under which our cultures are compelled to live in the laboratories can be deduced without great difficulty from different facts already mentioned. However, we thought it useful to add to the illustration of the milk culture another one reproducing an entirely "natural" occurrence. Figure 34 (Pl. F) was taken from a smear made directly from the content of a root nodule of red clover. The irregular, frequently branched

large forms<sup>1</sup> are passing over into the symplastic stage. Many bright gonidia, some deeply stained regenerative bodies, and a few normal slender rods are seen. This illustration should be compared with figures 26 and 27 of Plate E.

The two salt-water spirilla included in our experiments were also inclined to produce symplasm, globular and irregular regenerative bodies like the representatives of all other groups of bacteria. We have preferred, however, to show in figures 35 and 36 (Pl. F), which were made from a salt-beef-agar slant only 4 hours old, some facts which confirm and explain two observations made several decades ago. In 1887 Sorokin (19) published a preliminary communication upon his "new species" *Spirillum endoparagogenicum*, of which, so far as we know, a full description has never been given. His illustrations, reproduced in several textbooks, show clearly that he also found a budding bacterium without becoming aware of this fact. That the bright granules contained in the large spirilla and budding out of it, forming new small rods and spirilla, were not endospores, as the author asserts, seems to be beyond question. No test was made of their heat resistance, and in our opinion the fact that many of them were produced in the same cell proves sufficiently that they were gonidia. Their globular form is also much more in agreement with this opinion than with the assumption that they were endospores. Figure 35 (Pl. F) shows the same budding of our salt-water spirillum. Many of the irregular "involution" forms, so frequently observed with other spirilla, belong also to this type of growth. Furthermore, in figure 35 (Pl. F), as well as in figure 36 (Pl. F), several round regenerative bodies are reproduced, some of them being in the germinating stage. They are either dark-stained like those of other bacteria or they remain unstained when treated with aqueous anilin dyes. If such unstained forms are budding out of the end of a spirillum, as can be seen in the center and at the right side of figure 35 (Pl. F), we have apparently before us the same occurrence which was described by Prazmowski in 1880 (17, p. 43). We have not yet tested the heat resistance of these bodies. It is possible that they are parallel forms of the exospores found in the spore-forming L type of *B. azotobacter*. In the meantime they may be registered as unstained regenerative bodies. Some different types of germination are exhibited by the three regenerative bodies in figure 36 (Pl. F). The lower right part of figure 35 (Pl. F) contains several spirilla which may be in the conjunct stage. They are wound closely around each other, forming apparently one thick cell, only the end parts being separated. An analogous occurrence with *Spirochaeta obermeieri* has been recently observed by Levy

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<sup>1</sup> They have been called "bacteroids" by Branchorst because this author conceived the wrong idea that they were not bacteria, but cell products looking somewhat like bacteria. We are unable to understand how such an entirely incorrect term can still be used in modern scientific publications.

(12), who is also of the opinion that some "copulative" process takes place.

In future publications we will have to give more illustrations showing the different forms of the various kinds of regenerative bodies produced either directly by the different bacteria or by their symplasms. It seems as if such irregular, sometimes monstrous-looking formations as reproduced, for example, in figure 26 (Pl. E), are very constant and very characteristic for the species to which they belong. This is already well known in the case of *B. radicola*, and our figures 37 and 38 of Plate G may demonstrate the same fact in relation to *Micrococcus candidans*. The preparates were made from 6-day-old cultures in an ammonium-citrate solution. One strain (No. 44) had been isolated about six years ago from Russian black soil; the other (No. 45) nine years ago from evaporated milk. The characteristic appearance, as well as the uniformity of both illustrations, deserves our full attention.

Figures 39 and 40 (Pl. G) show the formation of well-stained gonidia by the yellow bacillus (No. 41) and by *B. fluorescens* (No. 40). In both cases the transformation of gonidia into regenerative bodies is clearly visible (cf. fig. 29 of Pl. E). Figure 39 (Pl. G) contains also in its lower left quarter some germinating regenerative bodies. The "budding" process is very conspicuous in this case, but the second picture makes it clear that the size of the gonidia frequently becomes so small that even a very careful observation of the stained preparate is hardly of any use. Besides, as many of these gonidia do not take the stain at all, some filtering experiments and the observation in the dark field seemed to be preferable.

#### FORMATION OF FILTERABLE GONIDIA

The formation of gonidia has been observed with all our different cultures, but whenever we saw a large number of gonidia in our preparates, there were always some, frequently many, just at the limit of visibility. It was to be expected that these would pass through Chamberland bougies. As our other experiments had shown that these gonidia are indeed living entities, some tests seemed to be of interest, especially in view of the many open questions concerning the occurrence and character of filterable vira.

The following cultures were used for making filtering tests:

No. 1 (*B. azotobacter*), 24 days old in a mannite-nitrate solution; No. 31 (*B. subtilis*), 11 days old, in ammonium-citrate-glycerin solution; No. 33 (*Tyrothrix tenuis*), No. 35 (*Bact. pneumoniae*), and No. 40 (*Bact. fluorescens*), each from a 2-day-old culture in ammonium-citrate-glycerin solution.

The filtrates were first tested under the microscope. Stained preparates had to show the absence of large forms. By the use of the dark field the small gonidia could easily be seen, some of them being actively

motile. These filtrates were then transferred to beef agar, beef broth, milk and blood serum. After incubating, the macroscopical appearance was the same in all cases. On the agar slope, especially on its lower moist part, a very scant, thin, slimy growth, somewhat resembling a very thin layer of small droplets of dew, became visible. A stained preparate from a 4-day-old slant clearly showed the gonidia germinating to minute rods (fig. 41 of Pl. G). The other substrates also gave no conspicuous growth.

The dark field proved more efficient in observing these almost invisible forms. Figure 42 (Pl. G), made from the same 4-day-old slant as figure 41, shows clearly that the filterable gonidia also form a symplasm in the same manner as the larger ones, which, in its turn, produces new small cells. In order to bring out more definitely the structure of this symplasm, it was necessary to make a very dark print, thereby obliterating several free granules which were also visible in the field.

These facts are in good agreement with the observations of various authors concerning filterable vira. In one of the latest publications along these lines Healy and Cott (9) described the filterable organism causing hog cholera as small globules or rods (0.2 to 0.3 $\mu$ ) when growing on ordinary media appearing in slimy clumps which are either well stained with aqueous dyes or are not stained at all.

As a regeneration of larger forms could in no case be observed on the substrates mentioned, we also transferred small quantities of filtrates of cultures 31, 35, and 40 into ammonium-citrate solution. Here a quick regeneration took place. After two days some sediment was already formed, which after shaking caused a distinct turbidity of the solution. Under the microscope in the stained preparate many pale, stained, small granules and minute rods were visible, as before, and also larger dark stained oval forms 0.5 to 1 $\mu$  broad, 0.75 to 1.5 $\mu$  long. These forms still differ considerably in their appearance from the normal rods of *B. subtilis* or *Bact. fluorescens*. They may be classified as regenerative bodies. That they will turn back entirely to the normal large vegetative cells is not doubtful, but this still remains to be tested experimentally. So far our tests have been repeated three times with *Bact. fluorescens* and twice with *B. subtilis*. The results were identical. For the filtration we used three different filters, which were controlled in each case by obtaining sterile filtrates from 1-day-old cultures of *B. subtilis* or *B. azotobacter* 1.

In carrying out experiments like these, however, another possibility of obtaining erroneous results must be kept in mind. Not only must the media be carefully prepared and sterilized but all glassware must be thoroughly treated with some cleaning fluid such as chromic acid, which destroys entirely all bacterial forms. The fact that mere sterilization is not sufficient is shown by the following test:

Particles of symplasm containing many regenerative bodies were carried from a mannite-nitrate solution to a similar medium and heated

in the autoclave for half an hour at 20 pounds' pressure. Even after this harsh treatment the microscopical picture was practically unchanged. As substrates rich in organic matter, such as beef agar, frequently contain symplasm and regenerative bodies resulting from former bacterial growth, they are especially liable to give misleading results.

#### DISCUSSION OF RESULTS

We hope that the facts mentioned in this preliminary communication will suffice to awake an adequate interest among our fellow bacteriologists, as there are numerous problems which now can be attacked successfully from this new standpoint. It is true that several authors before us have already spoken of the "life cycles" of bacteria. In most cases, however, they meant only the straightforward (not "cyclic") development, consisting in stretching and dividing of the cells, sometimes combined with the formation and germination of endospores. Fuhrmann (7, 8), who also wrote upon the "*Entwicklungskreise*" of bacteria, made some correct observations concerning the formation and further development of the gonidia. He was wrong, however, in concluding that these "granula" which he found in some spore-free bacteria were practically counterparts to the endospores in the "life cycle" of the spore-forming bacilli, and his opinion upon the "detritus" resulting from the disintegrating cells—namely, the symplasm—was far from being correct. In this direction Fokker (5) came much closer to the truth. It is not impossible, of course, that by a thorough sifting of the literature we shall discover some entirely forgotten author who was already on the right track. So far as we know now, only one bacteriologist has previously seen all the different stages of growth typical of the full life cycle of the bacteria. We refer to De Negri's important "*Untersuchungen zur Kenntnis der Corynebacterien*" (16), which appeared this spring, when we had just begun to prepare this paper for publication. A comparative study of the illustrations of his article and those of the present paper will be very instructive. He registered the following forms produced by the organism which causes the "malignant granulom:"

- Large globules (2.5 to 5.5 $\mu$ ) sometimes in sarcina form,  
eventually developing round or rodlike germs or buds. . . . . Our types A and B
- Large forms containing granules occasionally unstain-  
able . . . . . Our type C
- Crumbly agglomerations formed by large forms "melt-  
ing" together, which later give birth to new small  
forms . . . . . Our type D
- Small globules frequently in chains. . . . . Our type E
- Small short rods ( $\frac{3}{4}$  by 1 $\mu$ ), small slender rods ( $\frac{3}{4}$  by  
1 $\frac{1}{2}$  to 2 $\mu$ ), rods containing granules, curved rods, and  
rods showing racket form . . . . . Our type F
- Granulated threads dissolving into small globules . . . . . Our type G
- Entirely unstained bright rods . . . . . Our type H

Globular forms of different size sometimes showing a thin protruding rodlike form, irregular curved or clublike forms which later produce normal rods.....	Our type I
Budding large globules, budding and branching rods and threads.....	Our type K
Large rods and threads.....	Our type L
Pointed rods containing large granules.....	Our type M

This complete agreement is indeed very interesting, and as we ourselves have not worked with any representative of this group of organisms, De Negri's observations furnish a very welcome extension and confirmation of our statements concerning the life cycles of all bacteria. De Negri himself unfortunately failed to see that he was touching this general problem. He confined his studies almost exclusively to those corynebacteria causing "malignous granulom" and to some closely related forms. Therefore he was carried away to the entirely incorrect conclusion that those large budding forms were some kind of "blastomycetes," and the organism studied by him should be separated from the bacteria and placed among the Fungi Imperfecti. A comparative study of any of the common bacteria—for example, *B. subtilis*—would easily have prevented this serious error.

For *diagnostic* and *systematic* purposes a full knowledge of the life cycles of the bacteria will naturally be of the greatest importance. In our opinion the following morphological details should be studied in every case.

1. VEGETATIVE CELLS; FORMATION AND GERMINATION OF SPORES

- Spore-free and spore-bearing cells
- Arthrospores, formation and germination
- Endospores, formation and germination
- Exospores, formation and germination

2. CONJUNCTION OF DIFFERENT CELL TYPES

3. CONIDIA, formation and development

- Budding, liberating, germination, development in toto to regenerative bodies, to exospores, or to full-sized cells

4. SYMPLASM, formation by

- Spore-free cells
- Spore-bearing cells
- Arthrospores
- Endospores and exospores
- Regenerative bodies
- Conidia

5. REGENERATIVE BODIES

Formation by

- Spore-free and by spore-bearing cells
- Arthrospores, endospores, and exospores
- Conidia of different types
- Sytoplasm of different origin

Germination of the different types

Development in toto to vegetative cells or to spores

The improvement of the present situation is obvious. As the full life cycle of probably every species of bacteria can be studied without difficulty within a few weeks, provided suitable media are known and used for the experiment, we may hope that the time of reckless species-making will soon be ended. As said before, "good" species will win very much by such renewed and thorough study. The innumerable others, however, will have to take their modest place as links in those life cycles to which they really belong, or they will have to be canceled entirely. That the discovery of the conjunct and symplastic stage and further experimental studies upon it are of fundamental importance for reaching correct conclusions concerning species or varieties is beyond question.

Undoubtedly all our *physiological* studies will gain in much needed conformity and accuracy when established on the new broad morphological basis. It is to be hoped that such investigations now will also meet with more interest in botanical laboratories, where many of the general problems in bacteriology should be studied, as usually the time of agricultural and medical bacteriologists is completely taken up by their more specialized work. For instance, those curious but heretofore entirely unexplainable regular seasonal variations in the activity of bacteria in soils, quite frequently observed in Europe as well as in America during the last years, now seem to become explainable as a result of the seasonal effect upon the different modes of multiplication and propagation of the bacteria. A similar dependency on this factor then would exist as with other organisms. At least we can hardly consider it being merely an accidental coincidence that essentially the same annual curve, showing a maximum in spring and another one in autumn, is also followed by lower fresh-water algæ, where, as Transeau's careful investigations (20) have shown, the temporary prevalence of spore formation and of vegetative processes apparently represents the principal cause of these variations.

Concerning *pathological* problems, we readily admit that we are entirely laymen. However, we feel sure that this branch of bacteriology also would win considerably by making use of our observations. They show that Henri's (10) very interesting results obtained with *B. anthracis* could easily be duplicated with this or other pathogenic species simply by studying the relation of virulence and type of growth. That those abnormal looking and abnormally reacting forms obtained by the French author by the application of ultra-violet rays are nothing else than some of the regular though heretofore unknown types of growth of *B. anthracis* needs hardly be emphasized. Investigations upon the relations existing between pathogenic and nonpathogenic bacteria, as well as the experimental transformation of one type into the other, now undoubtedly become much more accessible and promising. The same holds true concerning the filterable vira. At least some of them are surely to be explained as nothing else than filterable gonidia of well-known bacteria.



That the discovery of the complete life cycles of the bacteria solves also some problems in *general biology* has been indicated earlier in this paper, when Fokker's theory (5) concerning the development of bacteria from granules in milk or blood was discussed. It may be added that also those much doubted and disputed strange observations of Bastian (2, 3), so persistently and extensively defended by their discoverer, now are coming under entirely new aspects. Readers interested in this question should compare especially Plates IV and V of Bastian's "Nature and Origin of Living Matter" (2) and those on Plates X and XI of his "Evolution of Life" (3) with our illustrations of the different kinds of symplasm and regenerative bodies. Figure 33 of the last-named plate (XI) looks practically like a reproduction of our prepareate shown in figure 14 (Pl. C). Bastian was wrong, of course, when he considered those large cells as being some torula form; but we know that De Negri (16) made the same mistake recently, which indeed is quite excusable. That the budding large cell in our figure 14 is really nothing else than a type of growth of a spore-forming bacillus will probably even now be doubted by one or the other bacteriologist. It is superfluous to point out that we do not share Bastian's ideas concerning abiogenesis. Our standpoint in this case is the same as in regard to Fokker's hypotheses. The weak points in Bastian's experiments are sufficiently clear to every expert reader of his books. This, however, should not lead to discarding indiscriminately all his undoubtedly carefully made microscopical observations.

It goes without saying that we will readily furnish subcultures of the strains used in our studies to everyone who asks for them. But it probably would be still more interesting and surprising to our fellow bacteriologists if they would make some investigations with their own well-known stock cultures along the lines discussed in the foregoing pages. Even a renewed microscopical study of old stained prepareates may become very instructive. For example, the senior author also did not know that for more than 11 years he had in his collection, patiently waiting to be photographed, that fine prepareate now shown in figure 6 (Pl. A) until, as stated before, he decided to take down his "theoretical blinders." We have already mentioned that a careful study of the illustrations contained in our daily used textbooks will now reveal several things which we were so very well trained not to see. Certainly the German philosopher Lichtenberg made a very wise remark when he said:

Was jederman für ausgemacht hält, verdient am meisten untersucht zu werden.

#### SUMMARY

A comparative study of 42 strains of bacteria has shown that the life cycles of these organisms are not less complicated than those of other micro-organisms. As representatives of practically all groups of bacteria have been tested and all, without exception, behaved essentially in the

same manner, in all probability analogous results may be expected with all species of bacteria.

All bacteria studied live alternately in an organized and in an amorphous stage. The latter has been called the "symplastic" stage, because at this time the living matter previously inclosed in the separate cells undergoes a thorough mixing either by a complete disintegration of cell wall, as well as cell content, or by a "melting together" of the content of many cells which leave their empty cell walls behind them. In the first case a readily stainable, in the later case an unstainable "symplasm" is produced.

According to the different formation and quality of the symplasm the development of new individual cells from this stage follows various lines. In all cases at first "regenerative units" become visible. These increase in size, turning into "regenerative bodies," which later, either by germinating or by stretching, become cells of normal shape. In some cases the regenerative bodies also return temporarily into the symplastic stage.

Besides the formation of the symplasm, another mode of interaction between the plasmatic substances in bacteria cells has been observed, consisting of the direct union of two or more individual cells. This "conjunction" seems to be of no less general occurrence than the process first mentioned. The physiological significance remains to be studied.

All bacteria multiply not only by fission but also by the formation of "gonidia"; these usually become first regenerative bodies, or occasionally exospores. Sometimes the gonidia grow directly to full-sized cells. They, too, can enter the symplastic stage. The gonidia are either liberated by partial or complete dissolution of the cell wall or they develop while still united with their mother cell. In the latter case the cell wall either remains intact or it is pierced by the growing gonidia, which become either buds or branches.

Some of the gonidia are filterable. They also produce new bacteria either directly or after having entered the symplastic stage.

The life cycle of each species of bacteria studied is composed of several subcycles showing wide morphological and physiological differences. They are connected with each other by the symplastic stage. Direct changes from one subcycle into another occur, but they are rather rare exceptions. The transformation of spore-free into spore-forming bacteria seems to be dependent on the conditions acting upon the symplasm and regenerative bodies.

The discovery of the full life cycles of bacteria may be helpful in many directions. Systematic bacteriology now can be established on a firm experimental basis. Physiological studies will win considerably in conformity and accuracy when connected with morphological investigations along these new lines. Several problems in general biology are brought under more promising aspects. Agricultural bacteriology and medical also will derive much benefit.

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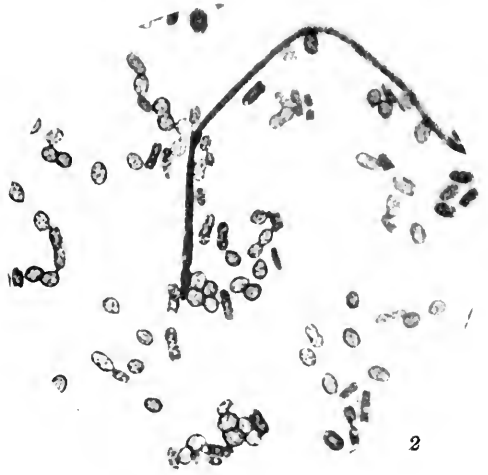
PLATE A

Magnification in all cases  $\times 1,000$ . Preparates stained with cold aqueous fuchsin unless otherwise noted.

- Fig. 1.—Azotobacter 11. Mannite-nitrate solution, 5 days old. Types A and La. Some cells in conjunction.
- Fig. 2.—Azotobacter 21. Contact prepare from a colony on mannite agar, 4 days old. Types A, L. Most cells in conjunction.
- Fig. 3.—Azotobacter 23. Contact prepare from a colony on mannite-agar, 4 days old. Types A, B, I, Kc, and many conjunct cells.
- Fig. 4.—Azotobacter 13. Mannite-nitrate solution, 17 days old. Type Kλ.
- Fig. 5.—Azotobacter 14. Mannite-nitrate solution, 5 days old. Type B forming I.
- Fig. 6.—Azotobacter 8. Beef bouillon. Type B forming types I and J.



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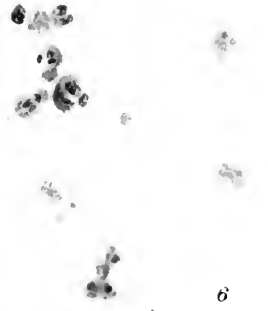
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PLATE B

Magnification in all cases  $\times 1,000$ . Preparates stained with cold aqueous fuchsin unless otherwise noted.

- Fig. 7.—Azotobacter 21. Mannite-agar colony, 4 days old. Type C forming types D and I.
- Fig. 8.—Azotobacter 22. Mannite-agar colony, 4 days old. Type C forming D also A in conjunction.
- Fig. 9.—Azotobacter 11. From a filter paper strip in mannite-peptone solution, 16 days old. Types A and B forming D.
- Fig. 10.—Azotobacter 3. Mannite-peptone solution, 24 days old. Types L and M forming D.
- Fig. 11.—Azotobacter 11. Mannite-peptone solution, 16 days old. Type D (stained) resulting from type C.
- Fig. 12.—Azotobacter 6. From condensation water of mannite-agar slant, 1 day old. Type D (unstained) containing regenerative units.



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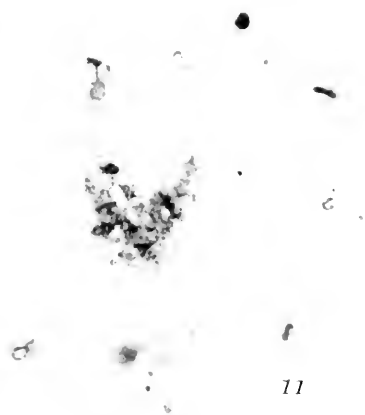
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PLATE C

Magnification in all cases  $\times 1,000$ . Preparates stained with cold aqueous fuchsin unless otherwise noted.

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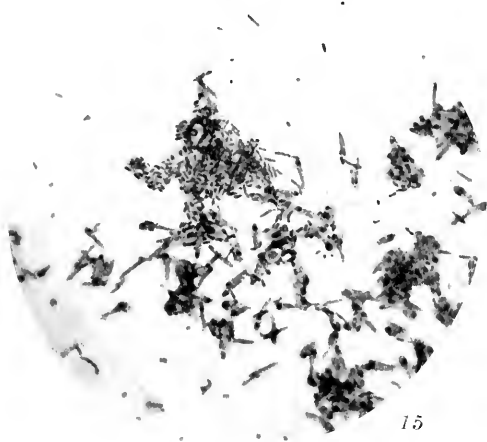
- Fig. 13.—Azotobacter 24. Mannite-nitrate solution kept 5 days after having been heated 1 minute at 96° C. Types I and F developing from D. Some I germinating in conjunct stage and inclining to form spores.
- Fig. 14.—Azotobacter 1. Mannite-nitrate solution, 10 days old. Types B, K $\beta$ , E, and F $\alpha$  developing from stained and unstained type D.
- Fig. 15.—Azotobacter 15. From condensation water of a mannite-nitrate agar slant, 2 days old. Types F $\alpha$  and F $\beta$  developing from type D.
- Fig. 16.—Azotobacter 17. Mannite-soil-extract agar, 2 months old. Types E, F $\alpha$ , K $\phi$ , and G developing from type D.
- Fig. 17.—Azotobacter 17. Mannite-nitrate agar, 10 days old. Prepare treated with hot aqueous fuchsine. Type G, partially dissolved; also K $\phi$ .
- Fig. 18.—Azotobacter 7. Mannite-soil-extract solution, 14 days old. Type H forming D.



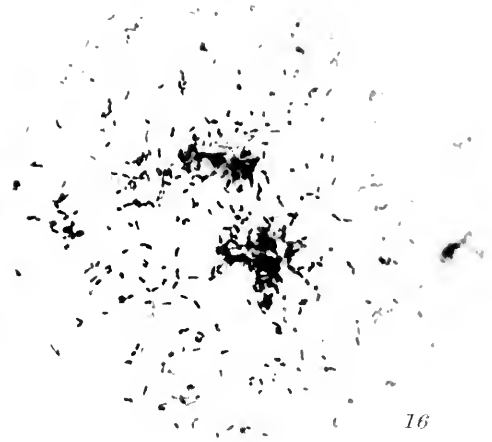
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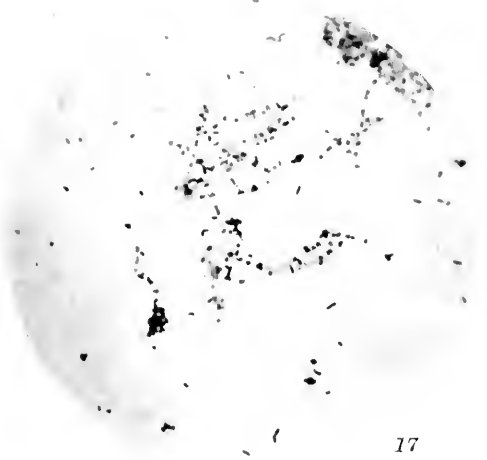
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PLATE D

Magnification in all cases  $\times 1,000$ . Preparates stained with cold aqueous fuchsin unless otherwise noted.

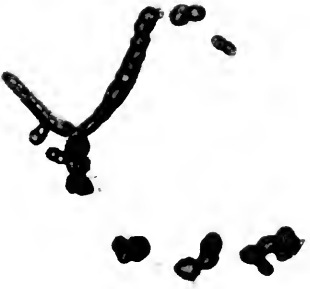
- Fig. 19.—Azotobacter 2. Mannite-nitrate agar, 23 days old. Spores forming type D.
- Fig. 20.—Azotobacter 2. Mannite-nitrate agar, 6 days old. Types L and F, endospores and exospores and dissolving of spores to type D.
- Fig. 21.—Azotobacter 1S. From a filter paper strip in mannite solution, 25 days old. Type L with gonidia, forming B (type Jλ).
- Fig. 22.—Azotobacter 7. Mannite-soil-extract agar, 2 months old. Types E and F forming B.
- Fig. 23.—Azotobacter 7. Mannite-soil-extract agar, 2 months old. Type B, formed by types E and F, germinating to type G.
- Fig. 24.—Azotobacter 7. Mannite-soil-extract agar, 2 months old. Type Kγ.



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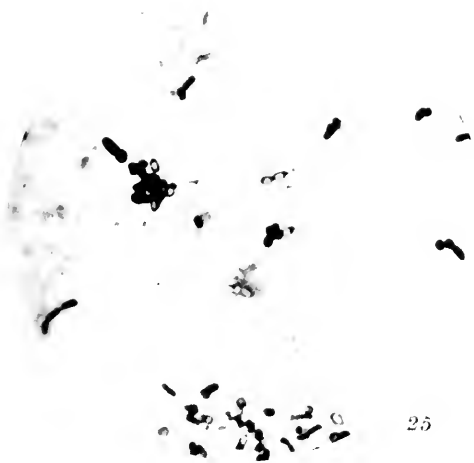


PLATE E

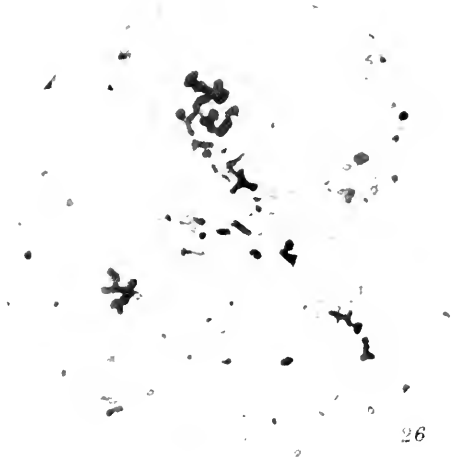
Magnification in all cases  $\times 1,000$ . Preparates stained with cold aqueous fuchsin unless otherwise noted.

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- Fig. 25.—*Bacillus subtilis* (No. 31). Beef agar, 2 days old. Types I and D formed by spores.
- Fig. 26.—*Bacillus subtilis* (No. 31). Beef agar, 6 days old. Formation of type I.
- Fig. 27.—*Bacillus subtilis* (No. 31). Beef agar, 8 days old. Type I forming H and stained D. Spores forming unstained type D.
- Fig. 28.—Yellow bacillus (No. 41). Peptone-glycerin solution, 2 days old. Type I germinating from D, stretching to type L.
- Fig. 29.—*Bacterium agaricum* (No. 49). Whey-yeast agar, 6 days old at 40° C. Types C, D, E, F, G, I, and K.
- Fig. 30.—*Bacterium fluorescens* (No. 40). Ammonium-citrate-glycerin solution, 11 days old. Types D and H.



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- Fig. 31.—*Sarcina flava* (No. 43). Beef agar, 1 day old. Type I in conjunction and forming D.
- Fig. 32.—*Streptococcus lactis* (No. 48). Peptone lactose solution, 5 days old. Type D, with regenerative units, forming type I.
- Fig. 33.—*Streptococcus lactis* (No. 48). Milk, 3 days old. Types D and I in casein.
- Fig. 34.—*Bacillus radicum* (No. 39). Types D and I. Prepare made from a root nodule in 1908.
- Fig. 35.—*Spirillum* sp. from Great Salt Lake (No. 46). Beef broth plus 3 per cent of sodium chlorid, 14 days old. Budding and branching forms; stained and unstained regenerative bodies. Some cells in conjunction.
- Fig. 36.—*Spirillum* sp. from Great Salt Lake (No. 46). Beef broth plus 3 per cent of sodium chlorid, 14 days old. Type I germinating.

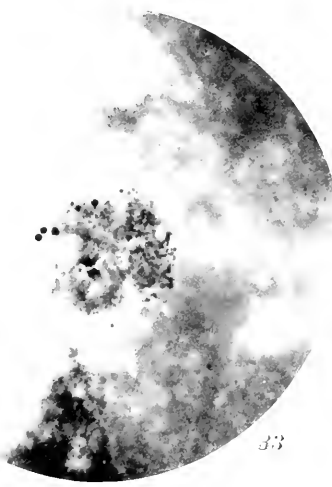




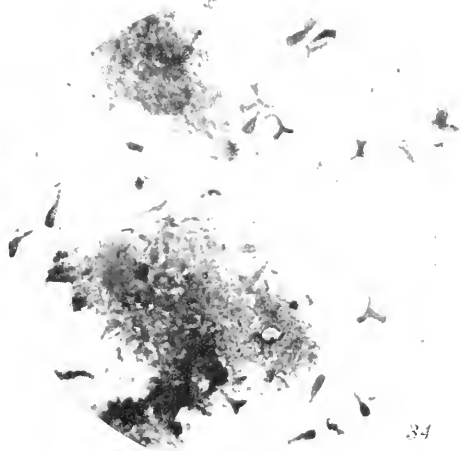
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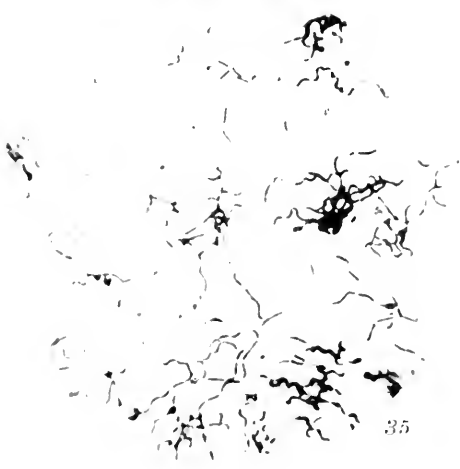
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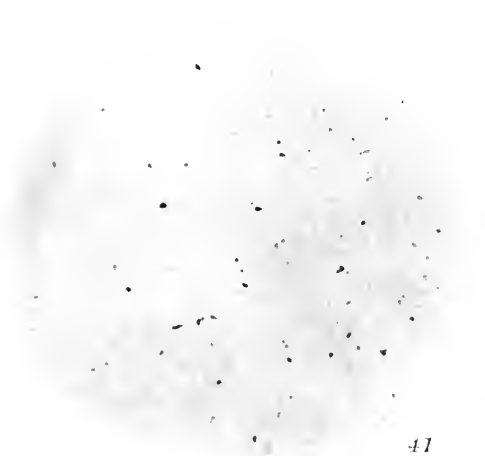
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PLATE G

Magnification in all cases  $\times 1,000$ . Preparates stained with cold aqueous fuchsin unless otherwise noted.

- Fig. 37.—*Micrococcus candicans* from soil (No. 45). Ammonium-citrate-glycerin solution, 6 days old. Irregular, thick-walled type I.
- Fig. 38.—*Micrococcus candicans* from milk (No. 44). Ammonium-citrate-glycerin solution, 2 days old. Irregular, thick-walled type I.
- Fig. 39.—Yellow bacillus (No. 41). Beef agar, 1 day old. Budding gonidia, formation and germination of type I.
- Fig. 40.—*Bacterium fluorescens* (No. 40). Ammonium-citrate-glycerin solution, 2 days old. Budding gonidia, formation and germination of type I.
- Fig. 41.—*Bacterium fluorescens* (No. 40). Beef agar, 4 days old. Filterable gonidia germinating.
- Fig. 42.—*Bacterium fluorescens* (No. 40). Beef agar, 4 days old. Types D and F formed by filterable gonidia. Dark field.





# A RESPIRATION CALORIMETER, PARTLY AUTOMATIC, FOR THE STUDY OF METABOLIC ACTIVITY OF SMALL MAGNITUDE

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## INTRODUCTION

A respiration calorimeter of the type of that described in a previous number of the *JOURNAL OF AGRICULTURAL RESEARCH*,<sup>1</sup> which is employed in the laboratory of the Office of Home Economics of the Department of Agriculture for the study of the metabolism of matter and energy in the human organism, is easily adapted to inquiries of similar character with other organisms. An apparatus much smaller than the one referred to has been developed in the same laboratory for use in the study of gaseous exchange and energy transformations of small magnitude and has been employed in investigations on the ripening of fruits and the wintering of bees. In fundamental principle this small respiration calorimeter is similar to the larger one mentioned above in that it combines a closed-circuit respiration apparatus and a continuous-flow water calorimeter. However, it differs from it in construction, having been modified in ways which make for ease of operation and for greater accuracy. Important changes have also been made in details, particularly with reference to its calorimetric features, the use of special devices for controlling and recording temperatures rendering it quite largely automatic in this respect. Brief accounts of this apparatus and of experimental work with it have been published,<sup>2</sup> but the details of construction and operation are given for the first time in the present article. A general view of the small respiration calorimeter is shown in Plate XCII.

## CONSTRUCTION OF THE RESPIRATION CHAMBER

The apparatus is devised so that chambers of different size or shape, constructed according to the varying needs of different investigations, can be substituted for each other. The chamber at present employed (Pl. XCIII) is 45 cm. square and 91 cm. deep, and has a total capacity of close to 185 liters. It was designed to accommodate a quantity of

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<sup>1</sup> Langworthy, C. F., and Milner, R. D. An improved respiration calorimeter for use in experiments with man. *In Jour. Agr. Research*, v. 5, no. 8, p. 299-347, pl. 30-36. 1915.

<sup>2</sup> ——— An improved form of respiration calorimeter for the study of problems of vegetable physiology. *In Orig. Com. 8th Internat. Cong. Appl. Chem.*, v. 18, sect. viiic, p. 229-236, 1 pl. [1912.]

——— A new respiration calorimeter for use in the study of problems of vegetable physiology. *In U. S. Dept. Agr. Yearbook 1911*, p. 491-504, pl. 65-67. 1912.

——— Some results obtained in studying ripening bananas with the respiration calorimeter. *In U. S. Dept. Agr. Yearbook 1912*, p. 293-308. 1913.

fruit sufficient for experimental purposes when stored in it under conditions approximating those of commercial practice, or otherwise, as desired. For example, a large bunch of bananas may be suspended in it from a removable cross of iron pipe the ends of which rest upon cleats fastened in the corners of the chamber near the top. Other cleats at different levels provide supports for shelves or for trays, baskets, or other containers in which the experimental material may be placed.

The walls of the chamber, which are of sheet copper 0.5 mm. thick, are attached to the inner side of a framework of hard-maple strips, experience having shown that using wood in place of metal lessens the possibility of error. The vertical strip in each corner of the frame is 3 cm. square, with the inner corner cut away on each side to a depth of 5 mm. to form a recess for the corner of the copper walls. At the top and bottom of the chamber, and midway between, the ends of cross strips 25 mm. square are joined to the posts so as to form a rigid supporting structure which is strong, though consisting of but little material. At the lower end each post extends 4 cm. below the bottom cross strip, to provide a leg for the structure. Elbows of stiff sheet copper, with one branch soldered to the outer surface of the copper wall and the other screwed to the framework, hold the copper firmly in place against the wooden frame.

At the top of the chamber is a close-fitting removable cover (Pl. XCIII) of sheet copper on a maple frame, with the metal projecting in a rim to hold the cover in place. The edge of the rim is bent down to fit into a groove in the flange formed by extending the copper side walls at the top to the outer edge of the maple frame. Wax melted into the groove seals the joint between the top and side walls when the cover is in place.

In the middle of the upper half of each of two opposite sides of the chamber is a framed opening 13 by 18 cm., forming a recess in which a pane of glass may be sealed (Pl. XCIII). These windows afford a view of the contents of the chamber and opportunity to watch the changes taking place. They may also be arranged so that either one may be opened during an experiment to remove a sample of the material under observation, if desired.

Another wall has a circular opening framed with a tube 9.5 cm. in diameter, in which is fitted a device called an "outlet" (see Pl. XCIII) which provides apertures for pipes conducting a ventilating current of air into and out of the chamber, for resistance thermometers providing passage for water entering and leaving the heat absorbers, for wires leading to electric-resistance thermometers inside the chamber, and for other purposes, as needed, all of which may be sealed in place. All openings into the chamber other than the windows are thus brought together in the one device, which is easily separated from the chamber so that the latter may be removed and another of different capacity substituted for it. During an experiment every joint in the chamber is air-tight.



As part of the arrangement described on page 716 for preventing the passage of heat through the walls, ceiling, and floor of the chamber, these surfaces are duplicated by sheet-copper top, bottom, and sides screwed to the outer edge of the wooden frame, the inner and the outer metal walls being thus separated by an air space 25 mm. across. There are openings in these walls for the windows and the "outlet" described above.

Surrounding the entire chamber, about 25 mm. from the outer metal wall, is a heat-insulating cover (Pl. XCIII) consisting of two layers of cork board 38 mm. thick, alternating with three layers of museum board 6 mm. thick, built up on wooden frames. The top, bottom, and side sections are built separately, and the several sections fit together with double-rabbeted joints, so that any one may be removed without regard to the others, or the entire cover may be instantly taken off. One section, as shown in Plate XCIII, is divided along the vertical median line, and all pipes and wires passing to the copper walls and to the "outlet" are brought out between the two halves of this section. The sections covering the two sides have openings to correspond with those in the walls of the calorimeter. The bottom section of the cover rests upon a substantial oak platform raised about 18 cm. from the floor of the laboratory.

#### DETERMINATION OF THE GASEOUS EXCHANGE

The respiration chamber in which the active material is confined is part of a closed air circuit through which a stream of air is constantly moving. The air which leaves the chamber is passed through purifying devices and returned again to the chamber. In the purifying devices the gaseous products resulting from the activity of the material in the chamber, which are carried out in the outgoing air, are absorbed. The purifying devices described below are those for the absorption of water vapor and carbon dioxide; but others could be substituted for these or connected with them if desired.

The quantities of water vapor and carbon dioxide carried from the chamber in a given period are shown by the changes in the weights of the absorbers during the period; and from these data, with due allowance for changes in the quantities of gases in the air of the chamber, the production of water vapor and carbon dioxide by the active material during the period is determined.

With a ventilation system of this type, as fast as any gas is removed from the air, other gas is introduced to maintain atmospheric pressure in the chamber. Usually oxygen is admitted, that being the gas consumed in respiration, as the term is commonly employed; but it is possible to vary the composition of the air at will, and if desired, to maintain an atmosphere of carbon dioxide or nitrogen or any other gas, which may be admitted to the system as oxygen is in the experiment as ordinarily conducted.

Oxygen to replace that consumed by the active material in the chamber is introduced into the air circuit from a reservoir of the gas. The quantity admitted is ascertained from the loss in weight of the container or by passing the gas through a meter. The amount admitted to the system and the change in the quantity of oxygen in the circulating air during a given period show the oxygen consumption of the material in the chamber.

A very light rubber bag on the end of a small copper tube extending from the chamber affords some variability in the capacity of the system, and by thus allowing for changes in the volume of gas present resulting from lack of uniformity in the rates of absorption of gas from the circulating air and the admission of other gas to it, or from changes in the temperature of the air within the chamber or in barometric pressure without, serves as an air-tension equalizer.

#### REMOVING WATER VAPOR AND CARBON DIOXID FROM THE AIR

The air withdrawn from the respiration chamber is forced first through sulphuric acid, which removes water vapor from it, and then through soda-lime, which removes carbon dioxide. The containers for the acid and the soda-lime, together with the air pump and the small electric motor by which it is actuated, are mounted on a stand with four shelves, called the absorber table (Pl. XCII). There are two parallel trains of absorbers on one shelf, one of which is in use while the units of the other are weighed and replenished.

The rotary air pump by which the circulation of air is maintained through the respiration chamber and the purifying devices has a capacity of approximately 100 c. c. per revolution, which is uniform for different rates of speed up to several hundred revolutions per minute. It is thus possible to vary the rate of ventilation of the chamber within a wide range simply by regulating the velocity of the pump, which is easily accomplished by means of a suitable rheostat to govern the speed of the motor which drives it. The rate of ventilation can be still further controlled, if desired, by means of a shunt in the air line between the inlet and outlet pipes of the air pump, with a valve to regulate the circulation through it. In the experiments for which the apparatus has thus far been used the former method has been sufficient, the pump being driven at a speed of 100 revolutions per minute, forcing 10 liters of air per minute through the system. With air ducts of brass pipe of 10-mm. bore the air flows in the circuit at very low pressure.

For absorbing water vapor from the circulating air, an acid bottle like that described for use with the large respiration calorimeter<sup>1</sup> but smaller in size has been found efficient. The bottle described by Williams,<sup>2</sup>

<sup>1</sup> Langworthy, C. F., and Milner, R. D. An improved respiration calorimeter for use in experiments with man. *In Jour. Agr. Research*, v. 5, no. 8, p. 306. 1915.

<sup>2</sup> Williams, H. B. Animal calorimetry. First paper. A small respiration calorimeter. *In Jour. Biol. Chem.*, v. 12, no. 3, p. 323. 1912.

which is shown in Plate XCII, has also proved quite satisfactory. A charge of 500 c. c. of sulphuric acid in either of these bottles will continue for several hours to remove all water vapor from air passing through at any rate maintained in the experiments thus far conducted with the apparatus, even though in some cases water vapor in the air is near the saturation point. The bottle and the acid weigh less than 2 kgm., and by means of a sensitive balance of 10 kgm. capacity the change in weight during a given period is ascertained to an accuracy of 0.05 gm.

Carbon dioxide is removed from the air which leaves the acid bottle by soda-lime in a large-sized U-tube of special design (Pl. XCII). Each arm of the U consists of glass tubing 23 cm. long and 75 mm. in diameter, and the two are joined at the bottom by glass tubing of 15-mm. bore bent in a semicircle, leaving a narrow space between them. The upper end of each arm of the U-tube is closed by a ground-glass stopper from the top of which projects a glass tube of 10-mm. bore bent at right angles. The bottom of the stopper is closed except for an aperture of 10 mm., and in the space within the stopper is cotton wool to prevent particles of soda-lime from leaving the tube in the outgoing air.

A piece of fine-mesh brass wire gauze is put on the bottom of each large tube to keep the bent portion empty, and each arm is then filled to the stopper with soda-lime in particles of about the size of a dried pea, approximately 2 kgm. of soda-lime being required to fill both arms. This amount of material when fresh will commonly absorb at least 100 gm. of carbon dioxide before it needs attention, which is indicated by the color of the soda-lime. The gray-colored material, which is somewhat moist in the fresh condition, becomes white with use, owing to both loss of moisture and absorption of carbon dioxide. When the moisture is entirely gone the efficiency of the soda-lime is low; but by passing moist air through the tube it may be restored to such an extent that the soda-lime may be used for at least one more period.

To catch the moisture given up by the soda-lime to the dry air coming from the first water absorber, the air leaving the U-tube is passed through another bottle of acid. Both the acid bottle and the U-tube, for which there is easily room on the pan of the balance by which the gain in weight of the absorbers is determined, are weighed together to find the quantity of carbon dioxide removed from the circulation of air. Their total weight is less than 5 kgm., and their change in weight is ascertained to an accuracy of 0.05 gm.

The air leaving the second acid bottle is passed through a trap of cotton wool (Pl. XCIII) to catch any spray that might be carried from the sulphuric acid by the moving air. The quantity of acid that leaves the absorber is so small that the trap need not be weighed.

## SUPPLYING OXYGEN TO THE AIR

The oxygen supplied to the chamber to replace that absorbed by the active material is obtained from a cylinder which contains the gas under pressure. It is admitted at such a rate that the apparent volume of gas in the chamber as indicated by the tension equalizer is relatively constant. The regulation may be by hand; or the tension equalizer may be arranged to cause a valve in the oxygen feed line to be opened or closed as the volume of gas in the system diminishes or increases. The small cylinder with the gas-pressure-reducing valve attached weighs less than 10 kgm., and changes in the weight of it may be ascertained to an accuracy of 0.05 gm., which means that the actual volume of the gas admitted is known to within 50 c. c. With regularity in the rate of admission of oxygen, other methods of determining the quantity, as by means of an accurate meter carefully calibrated, or by the filling and emptying of a calibrated spirometer, are suitable. In the latter case a sensitive spirometer could serve also as a tension equalizer.

## CHANGES IN THE COMPOSITION OF THE RESIDUAL AIR

At the beginning and the end of each experimental period a portion of the air leaving the rotary pump is shunted through a train of small absorbing devices <sup>1</sup> and then through an accurate meter, which stands on the top shelf of the absorber table. The air leaving the meter is restored to that in the main line returning to the chamber. The weight of each small absorber, which is less than 100 gm., is ascertained to an accuracy of 0.1 mgm. The quantities of water vapor and carbon dioxide in the measured sample of air, as shown by the increase in the weights of the small absorbers, represent very accurately those of the atmosphere of the chamber. With such material as ripening fruit in the chamber, any change in the composition of the atmosphere occurs so slowly that it has no appreciable effect on the air of the chamber during the period of taking the sample. A fan to stir the air is unnecessary, the total volume being small when the quantity of active material used for experimental purposes is inclosed in the chamber. The air is withdrawn from the chamber through a pipe terminating at the floor in a cross with both ends open, while air is returned to the chamber through a pipe opening near the ceiling. The circulating air thus traverses the full depth of the chamber. At the usual rate of ventilation the total volume of air in the system completes the circulation several times per hour.

When a sample of air is desired for the determination of the proportion of oxygen present, it is usually taken from that returning from the absorbers to the chamber, no oxygen being admitted from the cylinder to the system at the time. Ordinarily this determination is not necessary, since by properly accounting for the different products removed from and admitted to the ventilating system, the quantity of oxygen consumed from the atmosphere may be computed.

<sup>1</sup> Langworthy, C. F., and Milner, R. D. Op. cit., p. 311.

In the computation of the quantities of water vapor, carbon dioxide, and oxygen in the atmosphere of the chamber the actual volume of air in the chamber must be known, and this depends upon the capacity of the chamber under standard conditions of temperature ( $0^{\circ}\text{C}.$ ) and of barometric pressure (760 mm. of mercury) and the actual temperature and pressure of the air at the time the samples were taken. The barometric pressure of the air of the chamber, because of the tension equalizer, is always the same as that of the laboratory, which is determined to an accuracy of 0.01 mm. by means of a standardized barometer. The temperature of the air of the chamber is measured by means of an electric-resistance thermometer with the sensitive portion in the chamber and a temperature indicator outside. Either of two thermometers is available, one consisting of a single unit and the other of three units in series, which are modifications of the type of thermometer developed by Dickinson and Müller.<sup>1</sup> They are very sensitive and follow temperature changes rapidly. The single unit consists of a coil of nickel wire having a resistance of about 20 ohms at  $20^{\circ}\text{C}.$ , wound on a very thin strip of mica, placed between two similar strips, and inclosed in a flat case of thin copper pressed firmly against the mica. The portion of the case which incloses the coil is about 15 cm. in length, 13 mm. in width, and less than 1.5 mm. in thickness. The case terminates at the top in a short tube, through which the leads are extended to the resistance wire, being sealed in the tube with a hard wax to exclude moisture from the interior of the case. Each of the three units in series is constructed like the one just described, except that it has only one-third the total amount of resistance wire; hence, the unit is shorter, the other dimensions being the same.

The leads from the resistance thermometer coils pass through the "outlet" mentioned on page 704 and extend to a multiple-switch (Pl. XCIV), by which either the single or the triple thermometer may be connected with the temperature indicator, which does not appear in any of the views shown. The latter device consists of a Wheatstone bridge having a slide wire by which the bridge circuit may be kept in balance with the thermometer coils at any temperature between  $0^{\circ}$  and  $50^{\circ}\text{C}.$  The readings of the bridge scale, when translated into temperature by means of a calibration curve, show changes to  $0.1^{\circ}$ . The effect of the resistance of the thermometer leads and of their change in resistance, due to change in temperature, is neutralized by compensating leads from the opposite side of the bridge, so that the measurements by means of the bridge are of a high order of accuracy; although, because of the small volume of air in the chamber, absolute accuracy of these determinations is of less significance than in experiments with the larger respiration apparatus.<sup>2</sup>

<sup>1</sup> Dickinson, H. C., and Mueller, E. F. New calorimetric resistance thermometers. *In* U. S. Dept. Com., Bur. Standards Bul., v. 9, no. 4, p. 483-492, 2 fig. 1913.

<sup>2</sup> Langworthy, C. F., and Milner, R. D. *Op. cit.*, p. 312.

## DETERMINATION OF THE QUANTITY OF HEAT PRODUCED

The amount of heat resulting from the activity of the material in the respiration chamber is ascertained from determinations of (1) the quantity of latent heat in the water vapor of the outgoing water; (2) the quantity of sensible heat absorbed and carried away by water flowing in a coil of pipe in the chamber; and (3) the quantity of heat involved in changes in the temperature of the active material and of other objects in the chamber and also of the walls of the chamber. The gain or loss of sensible heat through the walls or in the ventilating current of air is prevented.

## LATENT HEAT

The quantity of water vapor carried from the chamber is determined from the gain in weight of the first sulphuric-acid bottle in the absorber train, as explained on page 706. If this quantity is multiplied by the factor 0.586, the product will be the number of Calories of latent heat at 20° C. carried from the chamber in the water vapor of the outgoing air.<sup>1</sup>

## SENSIBLE HEAT

Sensible heat emanating from the active material is removed from the chamber by a current of water flowing in a heat absorber, and the amount of heat thus removed in a given period is determined from the weight of water that flows through the absorber during the period and its temperature increase, with due allowance for the specific heat of the water at the mean temperature of the flow as compared with that at the temperature taken as standard. By controlling the rate at which water flows through the heat absorber, or the temperature at which it enters the absorber, the removal of heat is made to accord with its production, so that the temperature of the air of the chamber is kept as closely as possible to that which it is desired to maintain.

The heat absorber consists of 15 m. of copper tubing of 3-mm. bore in a double coil soldered to the upper and under surfaces of a piece of sheet metal 38 cm. square, with a double loop of pipe about 80 cm. long extending downward from each edge of the sheet. The absorber is removable and is slipped into position after the material under observation has been packed in the chamber. When in position, it is suspended with the sheet metal parallel to the ceiling of the chamber and about 25 mm. below it, with the double loops extending down the sides of the chamber and about 25 mm. from them.

## REGULATING THE RATE OF THE WATER FLOW

The water for the heat absorber flows from a constant level reservoir on a shelf above the calorimeter chamber, which is supplied from a tank on the lower shelf of the absorber table. The water that leaves the absorber is returned to the tank, from which it is raised again to the

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<sup>1</sup> Langworthy, C. F., and Milner, R. D. *Op. cit.*, p. 315.

reservoir by a small gear pump driven by the motor that actuates the air pump. The overflow from the reservoir also returns to the tank. The same water is used continuously in this manner to eliminate the difficulty resulting when water directly from the city main is passed through the system, owing to the presence of air dissolved in the water. If the temperature of the water is raised, the air escapes and collects in bubbles in the pipe and forms temporary obstructions that cause irregularity in the rate of flow of water through the absorber. With well-filtered water in the system a rate of flow as low as 5 liters per hour has been maintained with such uniformity that it would be sufficient to collect the water leaving the heat absorbers at intervals instead of continuously. Slight changes in the rate may be effected by the adjustment of a glass rod, with a long tapering end which passes through a constricted orifice in one end of a glass T-tube in the water line.

That the air of the chamber may be kept at any desired temperature, water is usually allowed to flow through the heat absorber at a constant rate and the temperature of the ingoing water is varied in accordance with the quantity of heat to be absorbed. To bring this temperature under control, the water is first cooled below that at which it is to be used, and then heated to the desired temperature. In these circumstances regulation of temperature is accomplished simply by variation in the amount of heating, which is easily controlled automatically.

#### REGULATING THE TEMPERATURE OF THE WATER FLOW

The water flowing from the reservoir to the heat absorber passes first through a pipe immersed in cold water to chill it, and then into a device called the preheater (Pl. XCIV) in which, by the conversion of electric current into heat in resistance coils inclosed in the water channel, the temperature of the water may be raised several degrees. The total heating effect of the device will increase the temperature of the water nearly  $6^{\circ}$  when the rate of flow is not over 500 c. c. per minute, and the heat may be added in small quantity. By this means the temperature of the water is raised near to that at which it is to enter the absorber. From this device, which is adjusted by hand, the water passes to the final heater (Pl. XCIV), which has a smaller capacity than the preheater, but is automatic and regulates the temperature within very narrow limits. The device is similar in some respects to that employed with the large calorimeter,<sup>1</sup> while in others it has been considerably simplified and improved.

The temperature of the water is raised or lowered by increasing or decreasing the electric current flowing in a coil of resistance wire immersed in the water. This is accomplished by adjusting the position of the sliding contact on a rheostat wound with resistance wire of graduated

<sup>1</sup> Langworthy, C. F., and Milner, R. D. *Op. cit.*, p. 319.

cross section in series with the heating coil. The contact is moved by a motor-driven mechanism, the movement being governed by the deflection of the pointer of a sensitive galvanometer incorporated in the mechanism. The terminals of the galvanometer are connected with a special Wheatstone bridge (the temperature indicator shown at *F* in Pl. XCIV), one arm of which is a resistance thermometer installed in the upper half of the water channel in the final heater, so that it is submerged in the water flowing past the heating coil in the lower half of the channel. The slide wire of the bridge is calibrated to cover a range of temperature from  $0^{\circ}$  to  $35^{\circ}$  C., and the scale of the bridge is graduated to  $0.1^{\circ}$ . If the temperature of the stream of water in which the thermometer is immersed differs  $0.05^{\circ}$  from that at which the pointer of the temperature indicator is set, the needle of the galvanometer is deflected, the direction in which it swings depending upon whether the temperature of the water is too high or too low, and the amplitude of the swing depending upon the amount of the difference in temperature. The effect of any deflection is a shift in the position of the contact on the rheostat, which alters the current in the heating coil and thereby varies its heating effect. This continues until the water is brought to the desired temperature. The extent of change in the temperature of the water at any single shift of the contact on the rheostat varies according to the magnitude of the deflection of the pointer, from one of an extremely small fraction of a degree to one of about  $0.1^{\circ}$ . The cam shaft by which the contact is shifted rotates in less than 3 seconds, so that alteration in the heating current may occur every 3 seconds if necessary. Thus, the temperature of the water may be changed very quickly; or, in other words, any variation in its temperature from that desired may be rapidly corrected.

From the final heater the water flows to the bottom of a bottle of about 3 liters' capacity (Pl. XCIV, *B*), filled with small pieces of pumice, from the top of which it flows to the heat absorber at a very steady temperature.

It has been stated on page 710 that one purpose of controlling the temperature of the ingoing water is to keep the temperature of the air within the chamber as constant as possible. The operator counteracts any tendency towards change in the temperature of the air by changing the setting on the indicator for the temperature of the water entering the heat absorber. By a slight modification in arrangement this could be made automatic. The resistance thermometer for the temperature of the air in the chamber could be connected with the temperature indicator in place of the thermometer in the final heater, so that whenever the temperature of the air varied from that set on the indicator the device for regulating the temperature of the water entering the heat absorber would be changed in such a manner as to correct it.



## MEASURING THE TEMPERATURE INCREASE

In the passage of the water through the heat absorber its temperature will increase according to its rate of flow and the quantity and activity of the material in the chamber. The accuracy with which the amount of heat carried from the chamber in the water current is measured depends upon that with which the temperature increase is determined. This is accomplished by means of electric-resistance thermometers and an automatic temperature recorder (Pl. XCV), in some respects similar to and in others different from that employed in connection with the large respiration calorimeter.<sup>1</sup>

In construction and characteristics the resistance thermometers are identical with those in the large calorimeter. They consist of two coils of platinum wire of equal resistance, which is about 25.5 ohms at 20° C., and have exactly the same coefficient of change in resistance with change in temperature, the resistance change of each being 0.1 ohm per degree. In each the resistance coil is encased in such a way that it is brought into intimate thermal contact with the flowing water and responds instantly and accurately to any change in its temperature. The water channels in which the resistance coils are installed are fitted into openings in the outlet described on page 704, to provide passage through the walls of the chamber for the ingoing and outgoing water, so that one coil acquires the temperature of the water just entering and the other that of the water just leaving the chamber.

The thermometers comprise two arms of a special Wheatstone bridge on opposite sides of a slide wire by which the bridge may be balanced for any inequality in the resistance of the two coils between 0.001 ohm and 0.2 ohm, resulting, respectively, from temperature differences of 0.01° and 2° between the ingoing and the outgoing water. The wire is calibrated so that temperature differences may be read directly from the scale. The total range of the instrument may be extended to indicate a difference as large as 5°. By means of resistance coils that may be connected in series with the slide wire as needed, the position of the balancing contact on the lower end of the wire may be made equivalent to a difference of 1°, 2°, or 3° between the thermometer coils, and the upper end 2° higher in each case.

The slide wire is incorporated in a mechanism which automatically balances the bridge for inequalities of resistance in the thermometers, and at the same time makes a graphic record of the balancing operations in terms of temperature difference and of time. The wire is mounted on the edge of a disk which is rotated to balance the bridge, while the balancing contact point remains fixed. The rotation of the disk, which is due to the action of one or the other of two cams on a shaft driven by

<sup>1</sup> Langworthy, C. F., and Milner, R. D. *Op. cit.*, p. 326.

a small electric motor, is governed by the deflection of the pointer of a very sensitive galvanometer, which is also incorporated in the mechanism, with its terminals connected with the Wheatstone bridge. When the bridge is in balance, the pointer remains at the zero position, and the slide wire is not moved; but any variation in the temperature of the water in either thermometer results in a change of resistance in the thermometer coils that upsets the balance of the bridge, the pointer swings to one side of the zero position or the other, according to the relation between the resistances of the opposite branches of the bridge, and the disk is turned in that direction in which the slide wire should be moved to restore the balance of the bridge. The amount of change in the position of the contact point on the slide wire is proportional to the magnitude of the swing of the pointer, which depends on the temperature difference in the thermometer coils. A difference of  $0.005^{\circ}$ , or even less, will upset the balance of the bridge sufficiently to cause a swing of the galvanometer pointer that will result in a movement of the disk. However large the temperature difference might be at any given instant, because of certain mechanical details connected with the mechanism for rotating the disk, stops are provided to limit the swing of the pointer either side of zero to that which would result from an inequality of resistance due to a difference of nearly  $0.2^{\circ}$  in the thermometer coils; but the cam shaft rotates every 5 seconds, and the disk may be moved that often if necessary; hence, the mechanism will keep the bridge balanced for inequalities resulting from any change of temperature difference in the ingoing and outgoing water up to  $2^{\circ}$  per minute.

The shaft on which the disk rotates also causes a pen to draw a line on ruled paper to show the direction and the distance that the slide wire had to be moved to balance the bridge. In the width of paper corresponding to the length of the slide wire that is equal to a difference of  $2^{\circ}$  there are 100 lines. The distance from line to line, which represents a temperature of  $0.02^{\circ}$ , is 2.5 mm. Hence, the temperature difference indicated by the position of the pen at any instant may be easily read to  $0.01^{\circ}$ .

The current in the bridge circuit is not sufficient to cause an increase in the temperature of the thermometers that will produce a movement of the pen even when the water is flowing through the thermometer at a rate much lower than the lowest that would be used with the apparatus.

A differential thermoelement is installed in the resistance thermometers so that the temperature difference of the water in the bulbs may be determined by means of a potentiometer as a check upon the measurement by the recorder. The Wheatstone bridge is provided with duplicate parts, which, by substitution, serve as means of checking the accuracy and constancy of the resistances of the bridge.

## CHANGE IN TEMPERATURE OF OBJECTS IN THE CHAMBER

Any change in the temperature of the walls of the chamber, or of the material confined within them, involves a quantity of heat for which allowance should be made in computing that produced in the chamber. For example, if the walls of the chamber are warmer at the end than at the beginning of the experiment, they have absorbed some of the heat that was produced in the chamber; while if they are cooler at the end of the experiment, some of their heat has been added to that in the chamber. The quantity of heat for which allowance must be made is computed from the change in the temperature of the walls and their hydrothermal equivalent—that is, the amount of heat involved per degree of temperature change in the walls.

The change in temperature is determined by electric-resistance thermometers devised for this apparatus. The resistance wire is wound in a flat coil about 5 cm. in diameter, which is firmly attached to one surface of a disk of stiff brass 55 mm. in diameter and 1.5 mm. thick. Through a hole in its center the disk is slipped over a short brass bolt projecting from the surface of the copper wall, so that by screwing a nut down on the bolt the disk may be clamped tightly against the wall, with the resistance wire between them. Between the wall and the wire are two or three layers of tinfoil to provide thermal contact in case of irregularity in the copper. The whole thermometer comprises 10 such coils, one for the top, one for the bottom, and one for the upper half and one for the lower half of each side. Each coil has a resistance of 45 ohms, but the 10 coils are connected in a series parallel arrangement to form a unit having a resistance of about 18 ohms at 20° C. The leads from this unit connect with the special switch and the Wheatstone bridge mentioned on page 709. The galvanometer will indicate a lack of balance due to a change of 0.05° in the temperature of the walls.

The most satisfactory data obtained in determining this factor indicate that for a change of 1° in the temperature of the walls the correction in the quantity of heat measured by the calorimeter would not exceed 1.5 Calories.

The correction involved in the change in temperature of the active material in the chamber is computed from the weight and specific heat of the material, and the temperature change as measured by an electric-resistance thermometer. One or the other of the two thermometers mentioned on page 709 is put into the mass of active material in such manner as to be in intimate contact with it. Tests with ripening fruit have shown that thermometers used in this manner indicate temperature change at least as accurately as a thermometer thrust into the flesh of one of the fruits.

## PREVENTING GAIN OR LOSS OF HEAT IN THE CHAMBER

Part of the arrangement for preventing increase or decrease in the amount of the heat in the chamber by the passage of heat through the metal walls consists in duplicating the side walls, ceiling, and floor of the chamber by parallel surfaces of sheet metal attached to the outside of the wooden frame, as explained on page 705. For convenience, the metal walls which actually confine the chamber—in this connection all six surfaces being considered walls—are designated the inner walls, while the corresponding surfaces on the outside of the frame are called the outer walls. If the temperature of the outer wall is regulated so as to keep it always like that of the inner wall, neither will transmit excess of heat to the other, and consequently there will be no gain or loss of heat through the walls.

The temperature of the outer wall is regulated by heating and cooling the air in the narrow space between the wall and the heat-insulating cover described on page 705. The air is cooled by chilled water flowing in a small-bore copper tube in the space and it is heated by the conversion of electric energy into heat in a resistance wire parallel with the pipe. The wire and the pipe for controlling the temperature of the side walls are shown in Plate XCIII. The chilled water flows through the pipe continuously at such a rate that the air in the space will be too cool when the heating effect of the electric current in the resistance wire is near its minimum, and the current in the resistance wire is regulated until the air is heated to the desired temperature. In these circumstances the temperature of the air may be raised or lowered simply by varying the current in the resistance wire, which is accomplished by adjusting a rheostat in series with the wire.

The rheostat is adjusted automatically by a motor-driven mechanism (Pl. XCV). The resistances of the rheostat are arranged in a circle about a shaft by which the contact point is shifted to vary the amount of resistance in series with the heating wire. The direction in which the shaft will turn depends upon the deflection of the pointer of a galvanometer mounted in the shifting mechanism, with its terminals connected to a Wheatstone bridge, two arms of which consist of electric resistance thermometers attached to the inner and the outer metal walls of the chamber. The coils of these thermometers are identical in construction with those described on page 715 and are similarly attached to the outer surface of the inner wall and the inner surface of the outer wall, the disks on the inner wall forming one arm and those on the outer wall the opposite arm of the bridge. The two units are identical in resistance at the same temperature, and with the galvanometer employed they form a very sensitive differential thermometer that is influenced by small changes in the thermal condition of the walls. If the temperature of the outer wall differs by as much as  $0.01^{\circ}$  from that of the inner wall, the

resistances of the two parts of the thermometer will differ accordingly, and the pointer of the galvanometer will be deflected, the direction of deflection depending upon whether the outer wall is warmer or cooler than the inner, and the contact point of the rheostat will be shifted so as to increase or decrease the heating of the outer wall and bring it again into thermal equilibrium with the inner wall.

Thermal equilibrium is maintained in the walls by sections rather than as a whole. The resistance coils on the inner and outer metal walls are grouped so that the top, the sides, and the bottom of the chamber each has its own differential thermometer; and provision is likewise made for heating and cooling each section independently, so that thermal conditions in each one may be regulated regardless of those in the others. Furthermore, in order that there shall be no excess of sensible heat carried into or out of the chamber in the ventilating current of air, the temperature of the air entering the chamber is regulated to accord with that of the air leaving. The units of a differential resistance thermometer are inclosed in the pipes carrying the ingoing and outgoing air through the walls of the chamber. Just before the pipe for incoming air reaches the calorimeter a short section of it is inclosed in an electric heating device to warm the air, while inside the same section of pipe is a small copper tube conducting chilled water to cool the air. As in the control of the temperature of the walls, the water is kept running continuously and the temperature of the air is regulated by varying the electric current in the heater surrounding the air pipe. The four rheostats controlling the currents for heating the top, sides, and bottom outer walls of the chamber, and the ingoing air are adjusted by the same mechanism (Pl. XCV), which operates them successively, any changes that are needed in a given rheostat being made once every four minutes.

The widest difference between the respiration calorimeter described in the present article and the larger one previously described in this journal<sup>1</sup> is in the method of preventing gain or loss of heat in the chamber. The devices described in the paragraphs above render this apparatus quite largely automatic in its operations as a calorimeter, whereas the other calorimeter is controlled mainly by hand.

By means of a switch, also operated by the mechanism, the galvanometer which governs the action of the regulating mechanism upon the rheostats is connected successively across each of the four Wheatstone bridges of which the differential thermometers are integral parts, each pair of thermometers being combined with its own ratio coils to form a bridge. These four sets of coils are mounted in the same case (Pl. XCV) in such a manner that the permanence of resistance of each may be easily tested. The coils in each pair may be transposed by changing the position of two plugs, whereupon the galvanometer deflection will alter

<sup>1</sup> Langworthy, C. F., and Milner, R. D. *Op. cit.*, p. 326.

if the coils differ in resistance. Moreover, the ratio coils of one bridge may be combined with those of either of the other three to form a test bridge, all four arms of which should have the same resistance. With a very sensitive galvanometer across the bridge thus formed, any inequality in the coils would be detected. It is assumed that if there is no deflection the coils have not changed in resistance, since it is hardly probable that all the coils would have changed equally. If any change should be detected, by varying the combinations it would be possible to determine which pair of coils was at fault. By shifting the point of contact of the battery lead on a short wire joining the two coils, equality of resistance may be restored when the changes are slight. No tests of this character have thus far indicated any need for change. Each bridge is also provided with a small variable shunt across a small resistance in series with one of the two differential thermometers to compensate for small inequalities in their resistances when at the same temperature.

#### TESTS OF THE ACCURACY OF THE RESPIRATION CALORIMETER

The accuracy with which it is possible under given conditions to measure the factors studied by means of the respiration calorimeter is shown by a comparison of the determined amounts of oxygen consumed and of carbon dioxide, water vapor, and heat produced upon combustion of ethyl hydroxid in the chamber with those which should result from the combustion as calculated from the quantity of alcohol burned and the percentage of ethyl hydroxid in it.

A burner inside the chamber is connected with a small-bore copper tube that passes through the "outlet" in the wall of the chamber. To the exterior end of this tube is attached a glass U-tube with one long arm into which alcohol for the burner is fed by dropping from a supply bottle which may be weighed at intervals to determine the quantity burned. To test the apparatus under conditions equivalent to those of experiments in which it is used, the alcohol must be burned at a very slow rate. Some difficulties were experienced at first in attempts to burn as little as 1 gm. per hour with complete combustion of the alcohol at a constant rate and with inappreciable loss by evaporation from the long arm of the U-tube. These were due in part to the fact that the opening in the "outlet" through which the alcohol tube passed is considerably above the level at which it is desired to have the combustion take place in the chamber. As a result of this condition, in all the tests thus far made the level at which the alcohol was maintained in the vertical tube was above that at which it was burned, attempts to feed the burner by siphon having proved unsuccessful. It was necessary to devise a burner which would overcome the effect of the pressure of the alcohol in the feed tube upon the rate of flow.

Burners of small-bore glass tubing of various diameters and with wicks of cotton, of glass wool, and of ignited asbestos, packed so as to allow

the alcohol to escape at the desired rate, were tried, but most of them were worthless because after combustion had continued a short time the flow of alcohol would begin to diminish and finally would be stopped entirely by material deposited in the top of the wick. This would occur even when the upper part of the wick was removed so that there was clear alcohol to a depth of 3 mm. or more below the flame. The phenomenon appeared to be associated with incomplete combustion of the alcohol, because whenever it occurred evidence that combustion was not complete could be found in the air of the chamber. That the material deposited in the wick was not in solution or in suspension in the alcohol was indicated by the fact that a sample of 100 gm. from the supply bottle when evaporated left a residue less than 0.1 mgm.

Some successful results were obtained with a burner of very thick wall and a bore of approximately 1 mm., with glass wool for a wick. When the glass wool was sufficiently tamped some pressure was necessary to force alcohol through it at the desired rate. The chief objection to this burner was the tendency of the thick tube to crack when the alcohol was lighted. Alcohol was fed from the supply bottle by dropping at such a rate that the level of the alcohol would remain at a mark on the long arm of the U-tube indicating the height which had been found by trial to produce sufficient pressure to keep the alcohol burning at the desired rate. This U-tube was of small bore to reduce the surface from which evaporation could take place, and the open end of the tube was nearly closed by the constricted nozzle of the tube from the supply bottle, leaving only a small space through which vapor could escape.

The results obtained with a burner consisting of two concentric small tubes and a wick of asbestos tape filling the narrow annular space between them were also quite satisfactory. No products of incomplete combustion were found in the air of the chamber when alcohol was burned in either of these burners at a rate even lower than 1 gm. per hour. The data in Table I show the results of two representative tests.

TABLE I.—Data obtained in the combustion of alcohol in the respiration calorimeter

Date.	Duration.	Weight of alcohol burned.	Water.		Carbon dioxide.		Oxygen.		Heat.		Respiratory quotient, CO <sub>2</sub> :O <sub>2</sub> .	
			Found.	Re-quired.	Found.	Re-quired.	Found.	Re-quired.	Found.	Re-quired.		
1914.	<i>Hours.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Cal.</i>	<i>Cal.</i>	
Jan. 21.....	5	9.20	9.7	10.7	15.7	16.2	17.4	17.7	61.2	60.4	0.655	
	5	9.22	10.1	10.7	16.0	16.2	17.3	17.7	61.0	60.5	0.673	
Jan. 27.....	10	18.42	19.8	21.4	31.7	32.4	34.7	35.4	122.2	120.9	0.663	
	11.75	10.34	11.8	11.9	16.7	17.2	18.4	18.7	.....	.....	0.660	

In the test of January 21 the alcohol was burned at a rate averaging slightly more than 1.8 gm. per hour. Almost exactly the same total quantity was burned in each of the two consecutive 5-hour periods com-

prising the test, and the determinations of carbon dioxide, oxygen, and heat in one period agree quite closely with those in the other, while the discrepancies between the quantities found and those required are for the most part small. The ratio of the volume of carbon dioxide produced to that of oxygen consumed in the test was 0.663, whereas the theoretical respiratory quotient for the combustion of alcohol is 0.667.

The only test in which less than 1 gm. of alcohol per hour was burned for any considerable period was that on January 27, which continued nearly 12 hours, at a rate of combustion averaging only 0.88 gm. per hour. There was a close agreement between the quantities computed and those determined in the measurement of gaseous exchange in this test also, but the heat production was not determined. These results are quite typical of all those obtained in tests of this character. In none of them have there been wider discrepancies than these shown between the measured and calculated quantities, the reasons for which were not ascertained and which could not have been avoided.

The accuracy with which heat generated in the calorimeter chamber can be determined is tested also by converting a known amount of electrical energy into heat in a resistance coil suspended in the chamber and measuring the heat with the calorimeter. In a test made on February 3, 1914, a current of 0.087 ampere was passed through a resistance coil of 1,680 ohms at an average pressure of 146.5 volts, generating

10.97 Calories of heat per hour according to the formula  $\frac{I^2R}{4.183} = \text{small}$

Calories per second at 20° C. The quantity of heat measured by the calorimeter was 11.04 Calories in the first hour and 11.08 Calories in the second hour of the 2-hour test. During the second hour the increase in the temperature of the water that flowed through the heat absorber in the chamber was measured by a potentiometer and the differential thermoelement installed in the resistance thermometers (see p. 713) as a check on the measurement by the thermometers themselves. The average temperature difference was 1.42° as indicated by the resistance thermometers and recorder and 1.40° by the thermoelement and potentiometer. The discrepancy between the computed and the measured amounts of heat in the second period of this test is wider than that in any other electric test with this respiration calorimeter. The closest agreement was that obtained in a test which continued only 1 hour, on November 2, 1912, in which the amount of heat computed to have been generated in the chamber was 7.54 Calories and that measured by the calorimeter was 7.56 Calories.

Both the electric and the alcohol tests indicate that measurements can be made with this respiration calorimeter to a high degree of accuracy.



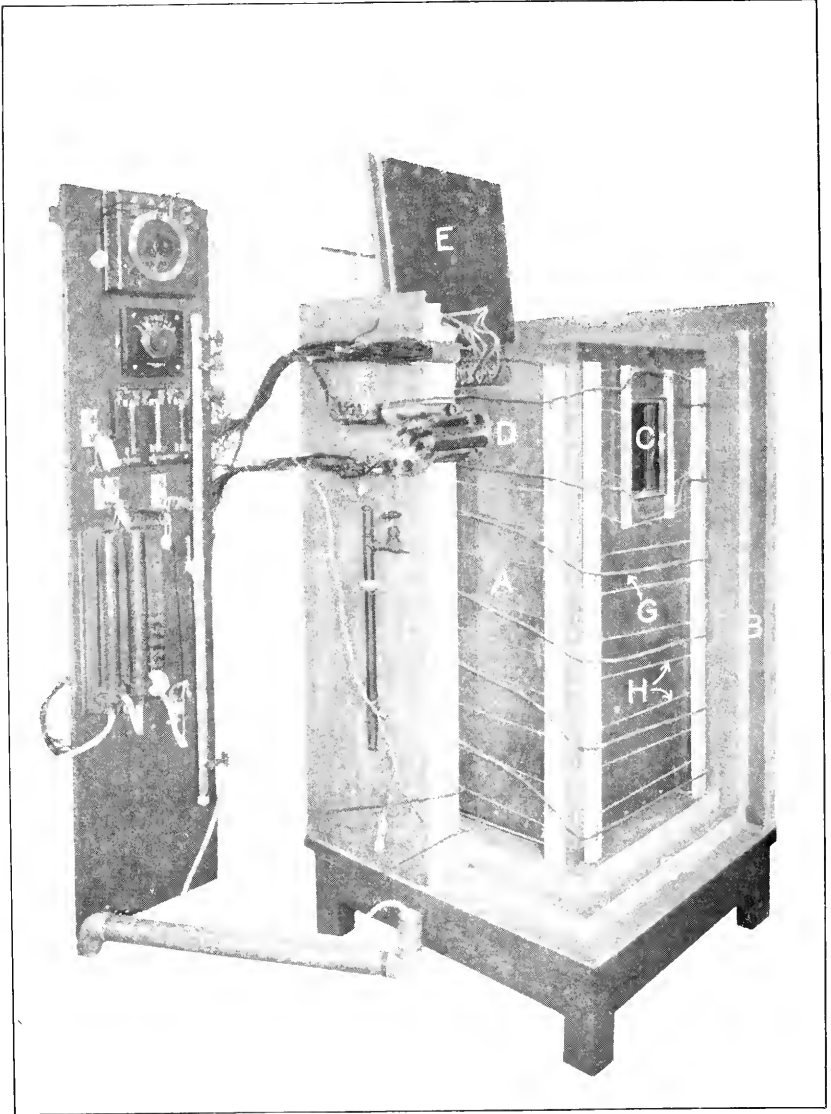


## PLATE XCII

### General view of the respiration calorimeter

*A*, Chamber inclosed in heat-insulating cover. *B*, Tension equalizer to maintain atmospheric pressure in the air of the chamber. *C*, Absorber table. *D*, Rotary pump to maintain air circulation. *E*, Motor to drive pump. *F*, Bottles containing sulphuric acid to remove water vapor from circulating air. *G*, Large U-tube, containing soda-lime to remove carbon dioxid from the air. *H*, Bottle containing sulphuric acid to catch the water vapor from the soda-lime. *I*, Bottle containing cotton to catch sulphuric acid vapor. *J*, Small absorbers for determining water vapor and carbon dioxid in residual air. *K*, Meter to measure the sample of residual air. *L*, Reservoir to maintain a constant pressure of water in the heat absorber in the chamber. *M*, Tank to catch water flowing from the heat absorber. *N*, Pump to raise water from the tank to the reservoir. *O*, Devices for automatically controlling and recording temperatures.





### PLATE XCIII

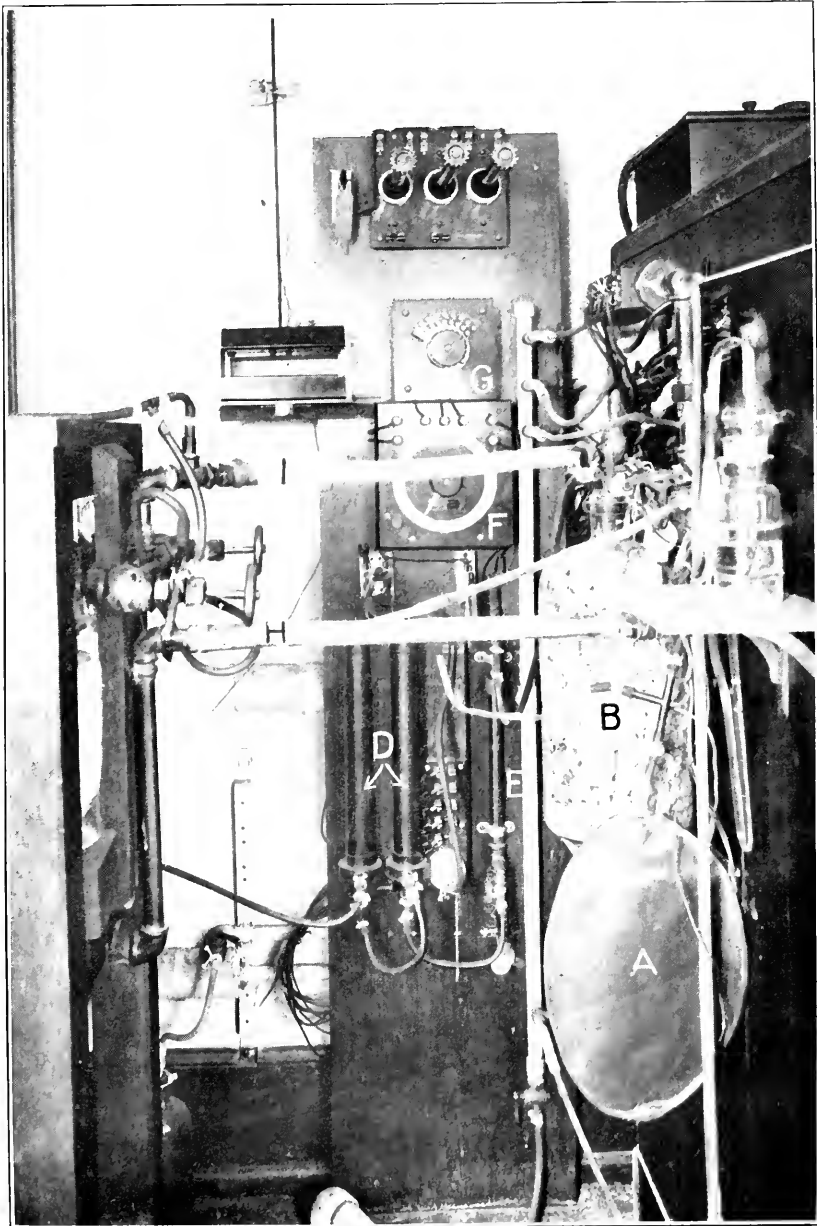
Chamber with part of outer covering removed

*A*, Double metal wall chamber. *B*, Heat-insulating outer cover. *C*, Window to chamber. *D*, Outlet providing passage for pipes, wires, etc., through the walls of the chamber. The exterior ends of the resistance thermometers for ingoing and outgoing water are seen projecting from the outlet. *E*, Removable top of chamber. *F*, Device for heating the air entering the respiration chamber. *G*, Small pipe carrying water for cooling the outer metal wall of the chamber. *H*, Electric-resistance wire carrying current for heating the outer wall.

## PLATE XCIV

Apparatus connected with the respiration calorimeter

*A*, Tension equalizer. *B*, Mixing bottle for equalizing the temperature of water entering the heat absorber. *C*, Device for heating air entering the respiration chamber. *D*, Preheater, and *E*, final heater, for raising the temperature of water entering the heat absorbers. There is an electric-heating coil in the lower half and an electric-resistance thermometer in the upper half of the final heater. *F*, Temperature indicator comprising part of the apparatus for controlling the temperature of the water entering the heat absorber. This device is connected with the resistance thermometer in the final heater and with the galvanometer in the controlling mechanism marked *B* in Plate XCV. *G*, Multiple-point switch for connecting the resistance thermometers for the metal walls and air of the chamber with the Wheatstone bridge for measuring their temperatures. *H*, Tube conducting air from the respiration chamber to the rotary air pump. *I*, Tube conducting air from the purifying devices to the respiration chamber.







## PLATE XCV

### Devices for controlling and recording temperatures

*A*, Mechanism for shifting the contact on the rheostats controlling the current for heating the outer walls of the calorimeter chamber and the ingoing air. The rheostats are almost entirely hidden at the rear of the case inclosing the mechanism. *B*, Ratio coils for the four bridges governing the action of the shifting mechanism *A* are combined in this box, together with means for checking the constancy of the resistance of the coils and for correcting slight inequalities in them and also to compensate for small differences in the pair of resistance thermometers forming the other arms of each bridge. *C*, Mechanism for shifting the contact on the rheostat controlling the current in the heating coil in the final heater, shown at *E* in Plate XCIV. The rheostat is below the case inclosing the shifting mechanism. *D*, Temperature-difference recorder (self-balancing Wheatstone bridge) for continuously recording the difference between the temperature of the water entering and that leaving the heat absorber. *E*, "Check box" containing the ratio coils of the bridge for temperature difference measurements and coils for extending the range of differences measured, with means for checking the constancy of the resistances of the coils and the accuracy of the recorder readings and also for compensating for slight differences in the resistance of the thermometer coils when they are at the same temperature.

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# JOURNAL OF AGRICULTURAL RESEARCH

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# JOURNAL OF AGRICULTURAL RESEARCH

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## MOTTLE-LEAF OF CITRUS TREES IN RELATION TO SOIL CONDITIONS

By LYMAN J. BRIGGS, *Biophysicist in Charge*, C. A. JENSEN, *Assistant in Plant Malnutrition*, and J. W. McLANE, *Laboratory Assistant, Biophysical Investigations, Bureau of Plant Industry*<sup>1</sup>

### INTRODUCTION

"Mottle-leaf" is a term applied in California to a mottled or spotted condition of the leaves of Citrus trees (Pl. XCVI). The affected portions of the leaf appear to be nearly or quite devoid of chlorophyll and are light yellow in color. In the first stages of the disease irregular spots several millimeters in diameter appear between the larger veins, usually midway between the midrib and the margin. The half of the leaf next to the tip is often first affected. In the more advanced stages, the spots are larger and more numerous, until finally the only chlorophyll remaining is confined to the midrib and the larger veins. The various stages are illustrated in Plate H. The condition is distinguished from what is generally termed "chlorosis" by the fact that the areas surrounding the yellowish spots retain their normal green color, at least until the spots embrace a large proportion of the leaf. The term "mottle-leaf" as here used is also to be understood as not including that type of functional disturbance sometimes found in Citrus leaves in which the midrib and veins are lighter in color than the surrounding tissue.

Mottle-leaf in its advanced stages is accompanied by a serious reduction in the yield and in the size and quality of the fruit. The foliage becomes thin and weak, with many very small leaves (Pl. XCVII); and the ends of the branches have a brushy appearance, owing to the development of numerous small weak twigs.

### DISTRIBUTION OF MOTTLE-LEAF

Mottle-leaf is at present quite widely distributed through the Citrus areas of California, but is, as a whole, worse in the southern sections of the

<sup>1</sup>The writers are indebted to the University of California Citrus Experiment Station and Graduate School of Tropical Agriculture at Riverside for many courtesies and facilities extended during the course of this work, and to Dr. H. L. Shantz and Mr. R. L. Piemeisel, of the Office of Alkali and Drought Resistant Plant Investigations, for their cooperation in the work preliminary to this investigation.

The writers also wish to express their obligation to the Citrus growers of the sections studied for their cooperation in supplying information regarding the field treatments of their groves and assistance in other ways.

State. It is not a new trouble in California, having been observed by some of the growers at least 15 years ago. During the last three or four years the mottling has become much more pronounced in the groves first affected. Other groves which are reported to have been relatively free from the trouble a few years ago are now badly mottled. There are many groves in the affected districts, however, that show little or no mottling at present.

#### FACTORS SUGGESTED AS CAUSAL AGENTS IN MOTTLING

The cause of mottling is a much disputed point. Various factors have been assigned as causal agents, such as an excess of lime, magnesium, or organic matter; a deficiency of lime, iron, or organic matter; frost; poor drainage, etc. Smith and Smith<sup>1</sup> conclude from their observations that the most prevalent and typical form of mottle-leaf is due to an irregular supply of moisture and plant food. No fungus or bacterium has yet been proved to be causally associated with mottle-leaf. Thomas,<sup>2</sup> however, has shown that the Citrus-root nematode (*Tylenchulus semipenetrans* Cobb) is widely distributed in districts in which mottle-leaf occurs, but is not invariably found on the roots of affected trees, and the extent to which mottling can be directly induced by such parasitism has not yet been determined.

One of the most striking features of mottle-leaf is the fact that the deficiency in chlorophyll is first in evidence in those portions of the leaf farthest removed from the midrib and largest veins; in other words, farthest from the main conducting channels of the leaf (Pi. H). This suggests a deficiency in the available supply of some substance essential in the formation of chlorophyll. The entire supply of this substance is apparently used by those portions of the leaf near the conducting channels, the supply being insufficient to reach the more remote portions of the leaf. That the disappearance of the chlorophyll is due to the absence of some essential constituent in the leaf rather than to the presence of some deleterious substance is also indicated by the fact that the chlorophyll next to the midrib and larger veins is the last to disappear. If the plant were absorbing something which reacted unfavorably on the chlorophyll, the effect of such absorption might be expected to be first in evidence nearest the veins. This analysis of the problem is to be considered simply as a working hypothesis which up to the present appears to accord with the observations.

The marked reduction in the yield of marketable fruit from badly mottled trees and the decrease in vigor led to the undertaking of a systematic survey of a number of groves in districts in which mottle-leaf

<sup>1</sup> Smith, R. E., and Smith, Elizabeth H. California plant diseases. Cal. Agr. Exp. Sta., Bul. 218, p. 1139. 1911.

<sup>2</sup> Thomas, E. E. A preliminary report of a nematode observed on citrus roots and its possible relation with the mottled appearance of citrus trees. Cal. Agr. Exp. Sta., Cir. 85, 14 p., 8 fig. 1913.

occurs with a view to determining the extent of its correlation with soil conditions. The results of these investigations form the subject of this paper.

#### FIELD SURVEY

As a basis for the investigation a field survey was made of about 175 orange and lemon groves near Riverside, Redlands, Highland, and Rialto, Cal.; a few groves were also examined in the Ontario, Pomona, and Azusa districts.

Ten or twelve representative trees were selected from each grove examined, usually a 10-acre block. The percentage of mottled leaves on each tree was determined by two men working independently. Soil samples were also taken near the same trees in 1-foot sections to a depth of 3 feet, the samples for a given foot section being combined. The fertilizer treatment of each grove and the cultivation and irrigation methods employed were also ascertained as accurately as the records of each grower would permit.

The soil samples representing each grove were promptly air-dried, and the organic carbon, "humus," total nitrogen, mineral carbonates, and bicarbonates determined in each sample. The moisture equivalent of each sample was also determined in order to compare the moisture retentiveness of the soils.

The limiting of the sampling to a depth of 3 feet was based upon the results of numerous observations, which showed that the feeding roots of orange and lemon trees do not as a rule extend much beyond this depth. The taproots, of course, go deeper when the soil conditions permit; but the feeding root system spreads out laterally near the surface, and this lateral feeding system does not ordinarily fully occupy the ground even to a depth of 3 feet. Excavations in the districts examined showed repeatedly that the main feeding root system was found usually from within a few inches of the surface to a depth of 18 to 24 inches.<sup>1</sup> Soil-moisture determinations in orange groves, to be presented in another paper, also showed that when the upper 3-foot layer dried out below the wilting coefficient, the tree could not get enough water to keep from wilting, even with available moisture immediately below this layer.

#### EXTRANEOUS FACTORS COMPLICATING THE CORRELATION OF MOTTLING WITH SOIL CONDITIONS

The study of the correlation between the degree of mottling and the general soil conditions of the groves is complicated by a number of extraneous factors. One of these is the kind of stock on which the selected buds are grafted. Other conditions being the same, a tree top on sour-orange stock is likely to show more mottling than one on sweet-

<sup>1</sup> Striking exceptions are, however, occasionally met with. Dr. H. J. Webber, Director of the University of California Citrus Experiment Station, informs the writers that in the Claremont section he has observed fine fibrous roots at a depth of 14 feet.

orange stock. In some instances also, tops on grapefruit stock were found to be more mottled than tops on sweet-orange seedlings. One specific instance of two adjoining navel-orange groves under the same ownership illustrates the case. One grove is on lemon stock, the other on sweet-orange stock. The trees on lemon stock showed 70 per cent of their leaves mottled, and those on the sweet-orange stock 50 per cent. Also, instances were found where individual orange trees on lemon stock were much more mottled than the surrounding trees on sweet-orange stock. As it is frequently impossible to obtain definite information about the stock used, this factor complicates the investigation. The physiological behavior of the buds on various stocks would be an interesting study in this connection.

Frosts and severe winds tend to increase mottling. After a strong, dry, north wind late in the fall of 1912 the leaves on the north side of the trees were more mottled than those on the opposite side. The new leaves put out later on the north side of the tree, however, were less mottled; and during the summer of 1914, the period covered by the field survey, the leaves on the south side of the trees were generally more mottled than those on the north side. Even in very severe cases of mottling, large healthy leaves are also often found in the center of the trees. This suggests that strong sunlight may increase mottling; and if so, the effectiveness of this agency would vary with the size of the trees and the closeness of planting.

Badly mottled trees cut back and rebudded on the stumps produce badly mottled new top growth, and the mottling persists unless the soil treatment is changed.

#### FERTILIZERS IN RELATION TO MOTTLING

The results of the field survey showed that groves which were plentifully supplied with organic material, either in the form of manures or green cover crops, were less mottled than those that had been fertilized entirely with commercial fertilizers. Several growers stated that they had cured mottle-leaf in limited areas by a liberal application of barnyard manure. It was also found in the case of all the groves included in this field study that each grove that had been fertilized with commercial fertilizers alone and kept under clean cultivation was badly mottled. This condition was especially marked in groves in which sodium nitrate had been employed for a number of years as the principal or only fertilizer. On the other hand, some groves that had received organic fertilizer were also badly mottled. This latter fact has discouraged many growers from using such material, especially manures, which usually have to be purchased and shipped in at high cost.

It was also observed that plowsole (an incipient hardpan just below the cultivated layer) frequently accompanied a badly mottled condition of the tree. Numerous observations have shown that the plowsole is a serious



obstacle in irrigation and gives rise to a droughty condition in the areas affected. The association of mottling with an inadequate soil-moisture supply appears in some instances to be clearly indicated. The relationship, so far as plowsole is concerned, is, however, complicated by the fact that plowsole is often, though not necessarily, associated with a low humus content.

#### RELATION OF MOTTLING TO YIELD

Badly mottled trees produce smaller fruits and a smaller number of fruits per tree than trees not mottled, and severely mottled branches produce less fruit buds. A slight mottling of the leaves does not appear to have any serious effect on the yield of fruit. The results of the field observations indicate that if less than 20 per cent of the leaves show mottling, the yield is not measurably decreased. The yields of oranges and lemons in the groves studied were obtained in most cases as far back as 1907, but the freezes of 1912 and 1913 proved such a disturbing factor both as regards yield and tree condition that it was not found possible to establish any relation between the yield and the mottling as determined in 1914.

#### SUSCEPTIBILITY OF DIFFERENT CITRUS TREES TO LEAF MOTTLING

Mixed groves of lemons and oranges were not found in the field survey, so that the relative mottling of the two species under the same cultural conditions could not be directly determined. From indirect comparison there appears to be no great difference in this respect. Grapefruit and tangerine mottle readily, but no opportunity was presented for a direct comparison with lemon or orange trees. There are few tangerines produced in the areas studied.

There seemed to be no difference between the Washington Navel and the Thompson Improved Navel so far as susceptibility to mottling was concerned. Where differences in mottling were found, it was also found that the two varieties were on different stocks. One mixed grove of Washington Navels and Valencias was studied, in which the two varieties were alternated in the same row, so that the conditions were the same for each. In this case both varieties were equally mottled.

#### RESULTS OF THE SOIL ANALYSES

##### METHODS OF SOIL ANALYSIS EMPLOYED

The total carbon was determined by boiling 20 gm. of soil with 50 or 75 c. c. of a mixture of sulphuric acid and potassium bichromate, using the larger amount with soils containing more than the average amount of organic matter. The acid mixture was made up in the proportion of 120 gm. of the bichromate to 1,000 c. c. of concentrated sulphuric acid. The carbon dioxide was absorbed in  $N\frac{3}{4}$  sodium hydrate in a bead tower and the whole of the hydrate solution removed and titrated.

The inorganic carbon (from mineral carbonates) was determined by boiling 20 gm. of soil with 50 c. c. of normal phosphoric acid under a partial vacuum of about 68 cm. of mercury, absorbing the carbon dioxide in  $N\frac{2}{3}$  sodium hydrate and titrating as in the case of total carbon. The phosphoric acid liberates the carbon dioxide in mineral carbonates or bicarbonates, but does not appear to attack appreciably the organic matter.

The humus was determined by removing the calcium from 10 gm. of soil with dilute hydrochloric acid (1 per cent), washing out the chlorids, extracting the soil with 500 c. c. of 4 per cent ammonia for 24 hours, and measuring the intensity of the humus color in a colorimeter against a standard humus solution.

The percentage of soluble bicarbonates was determined by shaking the soil with distilled water and allowing it to stand overnight. The clear supernatant liquid was pipetted off the following morning into a Jena flask, a few drops of phenolphthalein added, the flask covered with a watch glass and the solution boiled on a hot plate; while boiling, the red color was titrated out with  $N/10$  hydrochloric acid.

The total nitrogen was determined by the modified Kjeldahl method, which includes the nitrogen of nitrates.

#### ORANGE SOILS

The difficulties encountered in correlating tree growth with soil conditions as determined by a laboratory examination are generally recognized. The soil environment of a tree is by no means uniform, and a soil sample at best represents only the average soil condition, and wholly disregards the local variations in root distribution. In the present investigation the correlation between mottling and soil composition is further complicated by the fact that an orange or lemon tree is, generally speaking, slow in response to fertilizer stimuli under the method of orchard management prevailing in the area studied. An application of barnyard manure, for example, even when thoroughly worked into the soil, does not cause tree response until some time after the manure has decomposed. Under such circumstances a soil sample may not represent the soil conditions responsible for the condition of the trees at the time of sampling

TABLE I.—Analyses of orange-grove soils in California to a depth of 3 feet

No.	Percentage of—					Ratio of—			Moisture equivalent.	Trees.				
	Humus.	Total nitrogen.	Organic carbon.	Carbonates.	Bicarbonates.	Humus to lime.	Carbon to humus.	Nitrogen to humus.		Nitrogen to carbon.	Age.	Percent- age of mottled leaves.	Variety.	Location.
1	0.033	0	0.271	0.051	0.033	0.66	8.15	0	10.4	21	89	Valencia.	A. H. 74-2.	
2	0.033	0.034	0.267	0.051	0.033	0.66	8.00	1.01	10.4	21	73	do.	A. H. 74-3.	
3	0.041	0.037	0.244	0.062	0.038	0.67	5.90	0.88	13.6	21	88	do.	A. H. 52-4, exp. 1.	
4	0.042	0.034	0.247	0.065	0.038	0.63	5.90	0.82	13.2	21	90	do.	A. H. 52-4, exp. 2.	
5	0.055	0.038	0.255	0.075	0.038	0.73	4.65	0.70	14.1	21	93	do.	A. H. 52-4, exp. 3.	
6	0.047	0.036	0.230	0.060	0.037	1.58	2.65	0.55	11.1	16	58	Washington.	Trumgate.	
7	0.049	0.037	0.250	0.061	0.035	0.69	5.08	0.75	13.6	21	90	Valencia.	A. H. 52-4, exp. 4.	
8	0.059	0.038	0.250	0.062	0.036	0.72	4.70	0.64	13.6	21	90	do.	A. H. 52-4, exp. 5.	
10	0.057	0.034	0.189	0.052	0.033	3.70	3.33	0.60	10.7	21	76	Washington.	N. O. Co. Palmyr.	
11	0.069	0.032	0.144	0.044	0.031	2.02	3.08	0.46	12.6	21	93	do.	N. O. Co. Vict. 10	
12	0.067	0.035	0.272	0.054	0.038	1.37	3.76	0.71	14.9	13	91	Valencia.	A. H. 65-3, A. C.	
13	0.076	0.036	0.249	0.054	0.038	1.41	3.00	0.71	11.2	21	91	Thompson.	N. O. Co. Vict. 10	
14	0.076	0.038	0.202	0.054	0.034	1.19	3.18	0.71	14.7	15	9	Thompson.	L. V. W. B. Out. 5.	
15	0.078	0.036	0.202	0.054	0.034	1.19	3.18	0.71	14.7	15	9	do.	L. V. W. B. Out. 6.	
16	0.084	0.036	0.202	0.054	0.034	1.19	3.18	0.71	14.7	15	9	do.	N. O. Co. Vict. Hill.	
17	0.085	0.034	0.192	0.051	0.032	4.45	3.05	0.41	12.0	14	4	Thompson.	N. O. Co. Crouch.	
18	0.085	0.034	0.192	0.051	0.032	4.45	3.05	0.41	12.0	14	4	do.	N. O. Co. Crouch.	
19	0.086	0.040	0.204	0.051	0.032	1.95	2.22	0.47	14.3	14	30	Washington.	A. H. 68-1.	
20	0.095	0.036	0.184	0.048	0.032	5.15	3.10	0.38	10.5	21	66	Washington.	L. V. W. B. Palmyr.	
21	0.091	0.038	0.192	0.048	0.032	7.95	2.10	0.35	10.2	21	68	do.	L. V. W. B. Palmyr.	
22	0.093	0.039	0.195	0.042	0.036	8.45	2.01	0.30	11.5	21	55	Thompson.	L. V. W. B. Palmyr.	
23	0.099	0.041	0.185	0.052	0.034	1.70	3.91	0.57	11.1	23	73	Washington.	N. O. Co. Shoen. k. r.	
24	0.091	0.037	0.236	0.047	0.033	3.48	5.53	0.45	16.4	12	12	do.	N. O. Co. Vict. 20, exp. 10.	
25	0.093	0.041	0.203	0.048	0.034	3.48	5.53	0.45	16.4	12	12	do.	Elshire Rd.	
26	0.100	0.038	0.250	0.043	0.036	1.43	2.40	0.36	12.5	21	59	Valencia.	A. H. 86-1.	
27	0.100	0.038	0.250	0.043	0.036	1.43	2.40	0.36	12.5	21	59	do.	A. H. 86-1.	
28	0.102	0.032	0.249	0.055	0.025	1.02	2.37	0.50	11.2	15	39	Washington.	N. O. Co. Vict.	
29	0.102	0.041	0.228	0.059	0.033	1.06	2.18	0.42	10.3	13	28	do.	N. O. Co. Vict. 20, exp. 9.	
30	0.102	0.041	0.228	0.059	0.033	1.06	2.18	0.42	10.3	13	28	do.	N. O. Co. Vict. 20, exp. 9.	
31	0.103	0.036	0.184	0.049	0.030	3.71	1.70	0.34	11.5	12	82	W. and V.	Viv.	
32	0.104	0.039	0.185	0.048	0.027	5.68	1.77	0.20	10.8	12	82	Valencia.	L. V. W. B. Sun. Mt.	
33	0.110	0.031	0.203	0.040	0.023	10.90	1.83	0.35	11.8	12	87	do.	L. V. W. B. Sun. Mt.	
34	0.110	0.031	0.203	0.040	0.023	10.90	1.83	0.35	11.8	12	87	Washington.	A. H. 61-1.	
35	0.117	0.034	0.210	0.049	0.020	5.13	1.95	0.29	9.7	21	59	do.	L. V. W. B. Out. 8.	
36	0.113	0.031	0.210	0.049	0.020	5.13	1.95	0.29	9.7	21	59	do.	L. V. W. B. Out. 8.	
37	0.104	0.031	0.216	0.043	0.026	4.41	2.18	0.30	12.1	12	56	Washington.	Hae. Sun. Mt.	
38	0.115	0.043	0.282	0.048	0.024	6.30	2.45	0.37	12.0	12	52	do.	N. O. Co. Vict. 13.	
39	0.114	0.053	0.262	0.049	0.018	2.86	2.12	0.40	16.3	13	6	Valencia.	N. O. Co. Vict. 8.	
40	0.116	0.044	0.262	0.045	0.018	4.65	2.24	0.38	16.3	13	2	Washington.	N. O. Co. Vict. 17.	
41	0.119	0.044	0.280	0.051	0.017	1.41	2.36	0.37	16.4	13	Trace.	do.	N. O. Co. Vict. 17.	

TABLE I.—Analyses of orange-grove soils in California to a depth of 3 feet—Continued

No.	Percentage of—					Ratio of—			Moisture equivalent.	Trees.			
	Humus.	Total nitrogen.	Organic carbon.	Carbonates.	Bicarbonates.	Humus to lime.	Carbon to humus.	Nitrogen to humus.		Nitrogen to carbon.	Age.	Percent- age of mottled leaves.	Variety.
42	0.118	0.029	0.218	0.017	0.021	7.10	1.84	0.24	0.13	Years.	Trace	Washington	N. O. Co. Eur. 7.
43	0.122	0.034	0.033	0.028	0.033	4.46	1.63	0.28	0.17	12	18	Thompson	N. O. Co. Pach. 4.
44	0.128	0.038	0.253	0.039	0.024	3.27	1.98	0.30	0.15	13	57	Washington	Colton Ave. Sm.
45	0.07	0.036	0.270	0.068	0.030	1.42	2.38	0.37	0.14	15.5	55	do.	Colton Ave. Sm.
46	0.132	0.038	0.267	0.073	0.025	1.82	2.02	0.28	0.14	16.3	32	do.	N. O. Co. Vict. 12, exp. 3.
47	0.138	0.046	0.338	0.104	0.024	1.32	1.73	0.34	0.19	16.3	46	do.	N. O. Co. Vict. 12, exp. 5.
48	0.134	0.039	0.312	0.098	0.020	1.34	1.58	0.29	0.19	16.5	11	do.	N. O. Co. Vict. 12, exp. 6.
49	0.131	0.039	0.230	0.033	0.023	4.03	1.75	0.20	0.17	16.4	26	do.	N. O. Co. Vict. 12, exp. 8.
50	0.136	0.032	0.240	0.018	0.022	7.75	2.77	0.24	0.13	12.5	10	do.	N. O. Co. Viv. Low. 3.
51	0.146	0.038	0.315	0.028	0.023	5.28	2.17	0.26	0.12	11.8	32	do.	L. V. W. B., Oat. 7.
52	0.142	0.038	0.274	0.021	0.032	6.80	1.93	0.27	0.14	13.1	19	do.	N. O. Co. Vict. 1 and 2.
53	0.142	0.037	0.221	0.021	0.027	1.44	1.55	0.26	0.17	17.2	13	do.	N. O. Co. Vict. 26, exp. 7.
54	0.144	0.030	0.259	0.038	0.018	3.82	1.81	0.21	0.12	16.8	3	do.	N. O. Co. Eur. 12.
55	0.143	0.032	0.246	0.028	0.024	5.20	1.72	0.23	0.13	12.7	12	Thompson	N. O. Co. Viv., Low. 1.
56	0.141	0.037	0.244	0.018	0.022	7.67	1.73	0.26	0.15	12.7	8	do.	N. O. Co. Viv., Low. 2.
57	0.150	0.045	0.228	0.045	0.023	3.33	1.53	0.30	0.20	10.9	5	Washington	N. O. Co. Viv., Low. 4.
58	0.141	0.034	0.228	0.079	0.016	1.79	1.61	0.24	0.15	11.7	46	do.	N. O. Co. Flor.
59	0.143	0.038	0.255	0.036	0.022	1.77	1.78	0.26	0.15	11.0	53	do.	Bryn Mawr.
60	0.142	0.022	0.139	0.033	0.013	4.37	0.98	0.16	0.16	4.8	48	do.	E. H., Redlands.
61	0.156	0.033	0.289	0.019	0.019	8.16	1.85	0.21	0.12	11.8	58	Thompson	L. V. W. B., Dix.
62	0.153	0.044	0.250	0.071	0.023	2.16	1.63	0.29	0.18	17.4	15	Washington	N. O. Co. Vict. 9.
63	0.159	0.043	0.273	0.074	0.022	2.14	1.71	0.22	0.16	16.5	15	do.	N. O. Co. Vict. 26, exp. 2.
64	0.156	0.034	0.243	0.029	0.018	5.35	1.55	0.22	0.14	15.3	12	do.	N. O. Co. Eur. 9.
65	0.154	0.034	0.260	0.047	0.029	3.30	1.69	0.22	0.13	10.5	50	do.	N. O. Co. Spring 2.
66	0.152	0.027	0.201	0.018	0.026	8.37	1.32	0.18	0.14	12.1	67	do.	Vivi.
67	0.159	0.036	0.278	0.147	0.031	1.08	1.75	0.22	0.13	16.6	56	do.	Colton Ave. Sm.
68	0.169	0.041	0.291	0.053	0.026	3.20	1.73	0.24	0.14	11.5	44	do.	L. V. W. B., Oat. 4.
69	0.162	0.028	0.193	0.023	0.021	6.94	1.19	0.17	0.14	9.7	42	do.	Vivi.
70	0.165	0.043	0.265	0.02	0.047	1.60	1.60	0.26	0.16	8.7	95	do.	Bryn Mawr.
71	0.163	0.047	0.309	0.045	0.027	3.62	1.89	0.29	0.15	12.4	8	do.	Bryn Mawr.
72	0.162	0.046	0.220	0.133	0.022	1.23	1.36	0.31	0.21	13.5	1	do.	Redlands.
73	0.177	0.055	0.248	0.132	0.013	1.35	1.39	0.21	0.15	15.4	1	do.	N. O. Co. Vict. 15.
74	0.174	0.045	0.230	0.148	0.027	1.17	1.32	0.26	0.19	17.2	67	do.	N. O. Co. Vict. 26, exp. 1.
75	0.171	0.042	0.260	0.164	0.024	1.64	1.52	0.24	0.16	16.6	12	do.	N. O. Co. Vict. 26, exp. 4.
76	0.178	0.047	0.245	0.142	0.022	1.45	1.38	0.27	0.18	8.9	12	do.	Redlands.
77	0.186	0.048	0.270	0.031	0.023	6.03	1.45	0.26	0.18	12.9	96	do.	Palm Ave., Redl.
78	0.188	0.037	0.195	0.063	0.014	56.50	1.04	0.20	0.19	14.1	99	do.	Bloomington.
79	0.182	0.040	0.350	0.020	0.025	8.95	1.93	0.22	0.11	14.1	64	Valencia	L. V. W. B., Dix.
80	0.184	0.032	0.250	0.026	0.022	6.46	1.35	0.17	0.13	9.3	40	Washington	N. O. Co. Spring 1.

81	.199	.644	.668	.037	.032	7-15	1-40	.23	.46	15-4	13	Trace	Thompson	N. O. Co., Vict. 14.
82	.198	.646	.688	.058	.059	6-30	1-31	.20	.44	15-6	13	do	do	N. O. Co., Vict. 16.
83	.195	.638	.368	.039	.034	6-55	1-88	.30	.15	10-6	12	13	Washington	N. O. Co., Viv. Up. 2.
84	.194	.638	.368	.034	.034	6-55	1-88	.17	.15	15-2	12	14	do	N. O. Co., Viv. Up. 6.
85	.209	.641	.273	.033	.023	1-26	1-11	.15	.14	8-4	12	18	Grapfruit	N. O. Co., Viv. Low. 8.
86	.209	.641	.273	.033	.016	1-26	1-11	.32	.11	11-9	12	94	Navel	L. V. W. B., D.M. 1.
87	.215	.645	.274	.018	.018	1-26	1-29	.23	.17	12-7	12	36	do	Citrus Ave. Redd's.
88	.215	.645	.274	.018	.018	1-26	1-29	.43	.17	12-7	12	2	do	Bloomington.
89	.215	.645	.276	.009	.016	23-00	1-97	.14	.17	12-0	12	Trace	Washington	N. O. Co., Ehr. 6.
90	.218	.638	.358	.038	.137	11-81	1-37	.18	.13	13-0	12	65	do	L. V. W. B., D. 6.
91	.220	.639	.353	.031	.070	7-32	1-16	.18	.16	11-7	12	12	Grapefruit	N. O. Co., Viv. Up. 3.
92	.210	.639	.266	.039	.038	10-20	1-26	.18	.16	11-7	12	17	Washington	N. O. Co., Viv. Up. 5.
93	.221	.656	.209	.041	.041	5-13	1-15	.14	.17	13-8	12	4	Navel	Citrus Ave. Redd's.
94	.333	.638	.222	.010	.018	23-00	.95	.16	.17	9-1	Trace	Washington	do	Rock Highland.
95	.339	.650	.355	.035	.035	3-36	1-40	.21	.17	15-6	Trace	Navel	do	Rock Highland.
96	.332	.639	.278	.031	.034	11-25	1-40	.17	.16	10-3	12	0	Washington	L. V. W. B., Oct. 6.
97	.245	.644	.284	.024	.031	10-16	1-18	.18	.11	11-8	12	14	Thompson	N. O. Co., Viv. Up. 4.
98	.241	.637	.250	.011	.012	17-00	.83	.15	.18	11-8	Trace	Washington	do	Thomp. Highland.
99	.241	.624	.134	.017	.017	14-66	.55	.10	.18	4-7	None	Navel	do	Lin. Highland.
100	.271	.635	.266	.335	.032	8-1	.08	.13	.13	7-1	64	61	Washington	A. E. F., Highland.
101	.271	.635	.266	.335	.032	8-1	.08	.13	.13	7-1	64	64	Navel	Azusa.
102	.279	.627	.240	.021	.018	13-40	.86	.10	.12	6-1	Trace	Thompson	do	G. C. L., Highland.
103	.282	.627	.199	.073	.019	3-80	.71	.10	.14	8-9	39	39	Washington	N. O. Co., Eureka 5.
104	.287	.634	.277	.011	.025	26-40	.97	.12	.12	13-4	33	33	do	L. V. W. B., Oct. 1.
105	.302	.641	.265	.021	.033	14-40	.88	.13	.15	16-0	76	76	Valencia	Fr. Sun. Mt.
106	.313	.631	.297	.021	.021	4-20	.66	.10	.15	8-1	92	92	Navel	Azusa.
107	.333	.649	.428	.165	.038	3-17	1-22	.15	.12	9-2	12	12	Washington	L. V. W. B., Oct. 3.
108	.323	.642	.312	.011	.018	29-80	.97	.13	.13	7-9	12	12	Grapefruit	L. V. W. B., Sun. Mt.
109	.342	.658	.256	.068	.015	45-10	.84	.10	.10	7-7	84	84	Navel	Phy. Highland.
110	.346	.627	.273	.034	.016	10-10	.79	.08	.10	9-7	55	55	do	R. S. T., Highland.
111	.342	.638	.310	.104	.017	1-76	.88	.11	.12	16-7	53	53	Washington	Fl. Highland.
112	.353	.640	.281	.035	.013	2-28	.77	.11	.14	9-1	71	71	do	A. E. F., Highland.
113	.373	.640	.268	.104	.023	4-20	.80	.11	.14	9-8	70	70	do	Azusa.
114	.378	.633	.252	.009	.014	42-00	.75	.09	.12	8-9	10	10	Valencia	L. V. W. B., Sun. Mt.
115	.372	.612	.300	.012	.015	32-00	.82	.07	.10	7-5	None	Nursery	do	R. S. T., Highland.
116	.425	.643	.337	.016	.010	27-00	.80	.10	.13	8-2	None	Navel	do	Pomona.
117	.431	.645	.499	.013	.022	17-30	.98	.09	.09	10-9	None	Nursery	do	R. S. T., Highland.
118	.474	.641	.462	.026	.012	38-00	.98	.10	.09	8-1	None	Navel	do	Lock. Highland.
119	.503	.635	.437	.025	.018	20-00	.85	.08	.13	10-8	12	12	Washington	Rock. Highland.
120	.506	.642	.332	.011	.015	46-50	.66	.08	.10	8-7	None	Navel	do	L. V. W. B., Oct. 2.
121	.524	.633	.534	.033	.017	16-00	.98	.10	.10	13-0	None	do	do	Ontario.
122	.557	.652	.589	.018	.013	30-60	1-05	.09	.09	7-9	None	do	do	R. S. T., Highland.
123	.584	.638	.287	.115	.018	5-10	.49	.07	.13	10-8	19	19	Washington	Azusa.
124	.639	.640	.331	.057	.025	11-20	.52	.06	.12	7-1	None	Navel	do	Do.
125	.670	.660	.516	.107	.021	6-30	.77	.09	.11	15-0	Trace	Valencia	do	Do.
126	.695	.667	.628	.107	.022	46-20	.91	.11	.11	21-2	90	90	do	Pomona.
127	.769	.637	.298	.036	.012	22-40	3-87	.05	.12	9-7	90	90	do	Pomona.

The results of the analyses of orange soils, together with the percentage of mottled leaves in each grove, are presented in Table I. In all cases, unless otherwise stated, the soil data given represent a sample 3 feet in depth. Each foot section to a depth of 3 feet was analyzed separately, but the results of the determinations on the individual foot sections disclose no relationships that are not equally well represented by the mean value. The analytical data from the orange groves were first considered in relation to soil type. While the soils around Redlands, Highlands, and Riverside differ to some extent in their physical characteristics, no correlation between mottle-leaf and soil type was in evidence. Furthermore, the fact that mottling seems to be about equally advanced on all the soil types of this area, other conditions, as age of grove, general treatment, etc., being the same, would indicate *prima facie* that the soil type is by no means a controlling factor. The results obtained from all the orange groves studied in the districts around Riverside, Redlands, Highland, and Rialto are therefore presented collectively. A few groves studied around Pomona, Ontario, and Azusa are not included in this grouping, since the soil conditions of these districts are quite different, in so far at least as the organic content is concerned.

To facilitate further comparison, the orange-grove data are grouped in Table II on the basis of the percentage of mottling. Each group represents the average of about 20 groves, so that each point on the accompanying graphs represents an average of about 60 separate determinations of a given factor, and approximately 200 mottling determinations.<sup>1</sup> The fact that mottling is not dependent upon the texture of the soil is again emphasized in this table, which shows that the moisture retentiveness of the several groups as measured by the moisture equivalent<sup>2</sup> is very nearly the same.

TABLE II.—Analysis of orange-grove soils near Riverside, Redlands, Highland, and Rialto, Cal., grouped according to percentage of mottled leaves, each group containing approximately 20 groves

Group.	Percentage of—					Ratio of—				Moisture equivalent.	Mottled leaves.
	Humus.	Total nitrogen.	Organic carbon.	Mineral carbonates.	Mineral bicarbonates.	Humus to lime.	Carbon to humus.	Nitrogen to humus.	Nitrogen to carbon.		
1.....	0.119	0.036	0.237	0.069	0.023	1.72	2.50	0.303	0.152	<i>Per cent.</i> 11.3	<i>Per cent.</i> 88
2.....	.142	.036	.256	.066	.024	2.15	1.53	.254	.141	12.4	64
3.....	.170	.039	.254	.093	.026	1.83	1.07	.229	.154	11.6	43
4.....	.165	.039	.255	.080	.027	2.06	1.65	.237	.153	12.6	19
5.....	.244	.039	.261	.068	.020	3.59	1.93	.159	.139	11.9	8
6.....	.204	.038	.263	.079	.028	2.58	1.78	.186	.144	12.8	1

<sup>1</sup> The ratios in Tables II and IV are calculated from the mean values of the measured factors.

<sup>2</sup> The moisture equivalent is a measure of the moisture retentiveness of a soil, and is numerically equal to the percentage of moisture which a given soil is able to retain in opposition to a centrifugal force 1,000 times that of gravity. The finer the soil particles the greater is the moisture equivalent.

RELATION OF "HUMUS" IN SOIL TO LEAF MOTTLING OF ORANGES

The relation of the percentage of leaf mottling to the percentage of humus in the soil is shown in figure 1, the humus being plotted as the abscissas and the mottling as ordinates. While the points by no means form a smooth curve, there is a very evident inverse relation, showing that a high humus content is correlated with a low percentage of mottling.

As already mentioned, some time is required for an orange or lemon tree to respond to an application of manure. Consequently, in cases where manure has been recently added to a grove a measurable increase in humus may result without sufficient time having elapsed for a leaf response. Furthermore, when a leaf is well advanced in mottling, it does not recuperate (except by special leaf treatment), but remains mottled until it drops. Hence, a new set of leaves must be grown before the mottling will disappear from the tree, although the first stage of mottling, especially in a young leaf, may disappear as the leaf grows, if conditions become favorable. With these facts in mind, one

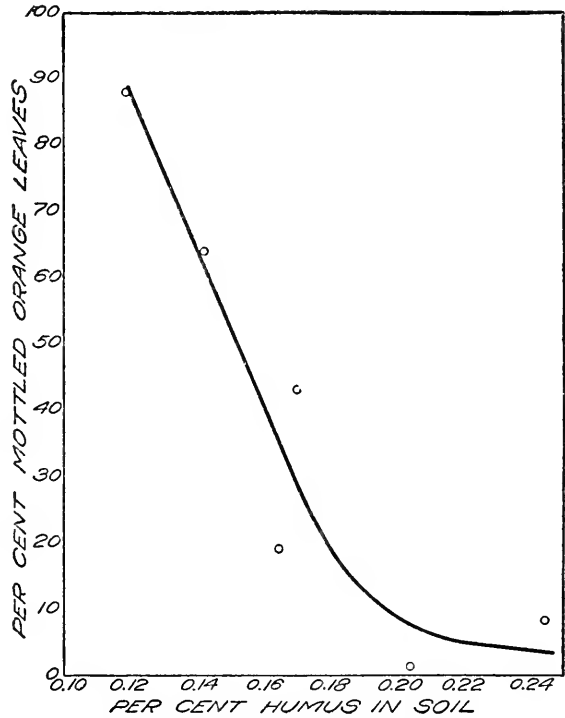


FIG. 1.—Graphical presentation of the relationship between humus content of soil and percentage of mottled orange leaves (from data in Table II).

would expect that the humus graph presented in figure 1 would show some inconsistencies, especially since other soil factors besides humus undoubtedly influence the nutrition of the tree.

The relationship between humus and mottling has been examined in more detail by the use of statistical methods. The form of the graph in figure 1 suggests an approximate hyperbolic relationship between humus content and mottling. In order to reduce the data to a suitable linear form for calculating the coefficient of correlation, the reciprocal of the humus content of each soil was calculated—that is, the number of grams

of soil required to include 1 gm. of humus. The coefficient of correlation between this quantity and the percentage of mottled leaves for all the orange groves included in the main group was found to be  $0.67 \pm 0.03$ . The association would be represented by the square of this quantity, or 0.45. In other words, approximately one-half of the mottling can be accounted for by the low humus content of the soil. This conclusion is reached from a consideration of the data by impartial statistical methods and is free from personal bias.

The failure of the trees in some cases to respond to manure appears to be due to the methods of cultivation and irrigation employed. It has been the general practice in California orange culture to maintain a deep dust mulch in the groves by cultivating frequently during the summer months. In fact, the cultivation which is carried on between irrigation periods, combined with the opening and closing of irrigation furrows, results in working the surface soil on an average of nearly once a week during the summer months. It is quite impossible for any effective root development to take place in this surface layer under such conditions. The roots are destroyed by the constant cultivation; and owing to the frequent stirring, the soil during the greater part of the time is entirely too dry for root development. Yet this is the part of the soil to which manure is applied, the usual practice being to disk the manure into the mulch. Even when the manure or a cover crop is plowed under, the plowing is often so shallow that the material turned under is within reach of the teeth of the cultivator. The result is, therefore, that the organic matter is partly disintegrated and lost without ever coming in contact with the feeding roots of the tree. Under such conditions it is not surprising that little benefit has resulted from the use of manure. It would be difficult to conceive a more effective method for the rapid destruction of the organic matter than the repeated stirring, moistening, and drying to which it is subjected in this deep surface mulch.

The difference in humus content between the soils of the badly mottled groves and those relatively free from mottling is about 0.1 per cent. This difference may at first sight appear small; but when expressed in terms of the weight of the soil, its magnitude becomes apparent. An acre of soil 3 feet in depth weighs approximately 10,000,000 pounds, so that a humus content of one-tenth of 1 per cent is equivalent to 10,000 pounds of humus. Data regarding the amount of humus formed from a ton of organic matter are not at present available, but manure would probably not often yield more than 10 per cent, or 200 pounds of "humus", or "*matière noire*", per ton. On the basis of this assumption it would require an application of at least 50 tons of manure per acre to bring the humus content of the badly mottled groves up to that of the groves relatively free from mottling.



Mention has already been made of the fact that the appearance of the mottled leaves indicates that the mottled Citrus tree is failing to secure something essential in the formation of chlorophyll. The association between mottling and low humus suggests that the missing substance may be some organic compound normally formed during the decomposition of organic matter in the soil or associated with the formation of humus, in which event the "humus" content would be indicative to some extent of the amount of this substance formed. Until further information is available in this connection, practical considerations point to the immediate enrichment of the humus content of the soil as the most promising specific for mottle-leaf.

#### RELATION OF MINERAL CARBONATES TO MOTTLING OF ORANGE TREES

The mineral carbonates in the soils of the area studied consist for the most part of calcium carbonate (limestone). The percentage is usually low (see Table I), although large deposits of limestone are found in some of the hills rising from the floor of the valley. No significant correlation was found to exist between the percentage of mineral carbonates and the percentage of mottled leaves (correlation coefficient =  $0.07 \pm 0.06$ ). In other words, there is no evidence that the amount of mineral carbonates within the limits found in these soils bears any relation to mottling. Most of the groves in the areas studied have not been limed, and where lime has been used, the amount applied has with few exceptions been so small as to be negligible in the determinations. For example, an application of a ton of limestone per acre would mean an increase of only two one-hundredths per cent when calculated on the weight of the soil to a depth of 3 feet. The effect of heavy applications of lime on mottling has not yet been definitely settled by properly controlled field experiments. This matter should furthermore not be confused with the evident beneficial effect of lime in improving the physical condition of some of the soils in the area studied.

#### RATIO OF HUMUS TO MINERAL CARBONATES AS AFFECTING MOTTLING OF ORANGE TREES

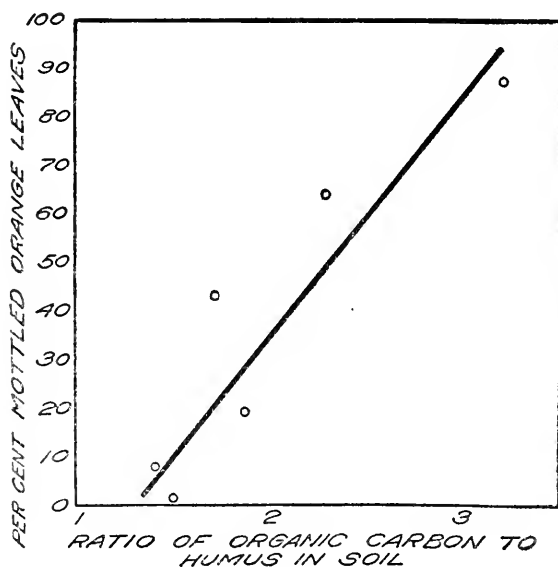
The ratio of humus to mineral carbonates in orange groves is plotted in figure 2 against the percentage of mottling. While the relationship is not marked, the mottling tends to diminish as the humus-lime ratio increases. The correlation between the reciprocal of this ratio and the mottling was computed and found to be  $0.17 \pm 0.06$ . Since no relationship was observed between the lime content of the soil and the percentage of mottling, it seems probable that the correlation observed in the case of the humus-lime ratio is dependent wholly on the humus correlation. The result indicates that the humus content of the soil should be taken

into consideration in applying lime and that such treatment would be more likely to be beneficial in the case of soils with a high humus content.

#### RELATION OF ORGANIC CARBON TO MOTTLING OF ORANGE LEAVES

The correlation between the total organic carbon in the soil and the leaf mottling is very low ( $-0.10 \pm 0.06$ ). Organic matter is not effective in nutrition until decomposition has set in, and the results indicate that the amount of those decomposition products effective in the control of mottle-leaf and available in the soil at a given time is not necessarily proportional to the total organic carbon present. The negative sign

of the correlation coefficient shows that the mottling tends to decrease as the organic matter increases.



RATIO OF ORGANIC CARBON TO HUMUS IN RELATION TO MOTTLING OF ORANGE LEAVES

The data presented in Table II for the six groups of orange groves show that an increase in the ratio of organic carbon to humus is accompanied by an increase in mottling (fig. 3). This relationship may be partly due to the fact that the mean organic

FIG. 2.—Graphical presentation of the relationship between the ratio of organic carbon to humus in the soil and the percentage of mottled orange leaves (from data in Table II).

content of the soils of the several groups is nearly the same throughout, although the reduction in mottling is accompanied by a slight increase in organic carbon.

A correlation of  $0.43 \pm 0.06$  was found between the organic carbon-humus ratio and the percentage of mottling. While this correlation may be partly associative, as in the case of the lime-humus ratio, the results indicate that it is important in the nutrition of the orange tree that the organic matter be decomposed, so far as possible, into humus, since the greater the proportion of humified organic matter, the smaller the percentage of mottling. This, of course, does not necessarily indicate that what we term "humus" is the most effective form of organic matter for promoting a healthy growth of orange leaves; but if a

soil can properly humify organic matter, the latter will apparently go through the decomposition stages most beneficial to the growth of the tree.

NITROGEN CONTENT AND MOTTLING

The total nitrogen content in the soil was surprisingly uniform regardless of grove conditions and soil types. The variation in total nitrogen within the limits found in the soils of the groves examined appears to bear no relation to the percentage of mottling (correlation coefficient =  $-0.02 \pm 0.06$ ). A part of the nitrogen is undoubtedly held

in a form not immediately available to the tree and in this respect is somewhat analogous to the total organic carbon in the soil. When the orange soils are grouped on the basis of mottling, as in Table II, the two most badly mottled groups show the lowest average nitrogen content, but the differences are so small as to have little significance. The other groups will be seen from Table II

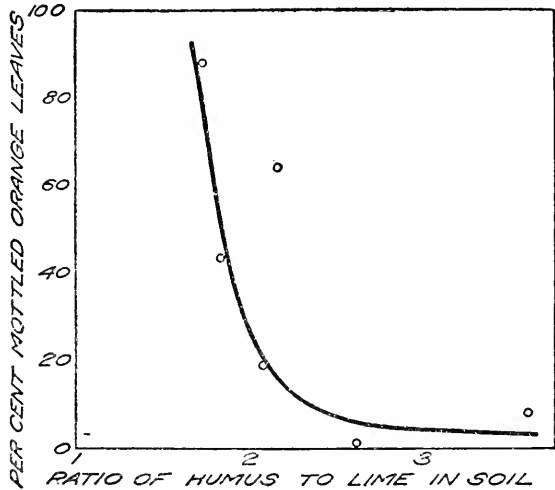


FIG. 3.—Graphical presentation of the relationship between the ratio of humus to lime in the soil and the percentage of mottled leaves (from data in Table II).

to have practically the same average nitrogen content. Such relationship as exists may be due to the fact that part of the total nitrogen is combined as "humus," so that the low humus soils would be lower in nitrogen.<sup>1</sup>

LEMON SOILS

The field studies of the lemon groves were carried on in a manner similar to that of the orange groves. The lemon groves selected all belong to the same company and constitute the principal lemon groves in the Riverside district. The data for the individual groves are presented in Table III and are grouped on the basis of mottling in Table IV.

<sup>1</sup> For data regarding the nitrate content of the soils of the Riverside area, see Kellerman, K. F., and Wright, R. C. Relation of bacterial transformations of soil nitrogen to nutrition of citrus plants. *In* Jour. Agr. Research, v. 2, no. 2, p. 101-113, 7 fig. 1914.

TABLE III.—Analyses of lemon-grove soils in California to a depth of 3 feet

No.	Percentage of—					Ratio of—				Moisture equivalent.	Trees.		
	Humus.	Total nitrogen.	Organic carbon.	Carbonates.	Bicarbonates.	Humus to lime.	Carbon to humus.	Nitrogen to humus.	Nitrogen to carbon.		Age.	Percentage of mottled leaves.	Location.
1..	0.044	0.031	0.265	0.041	0.028	1.08	6.00	0.70	0.12	11.5	20	88	A. H. 32 -36
2..	.045	.035	.275	.058	.025	.77	6.14	.78	.13	10.5	20	93	A. H. 32 -40
3..	.047	.031	.260	.009	.026	.68	5.48	.68	.12	12.6	21	44	A. H. 80 -6
4..	.058	.030	.215	.032	.019	1.81	3.72	.53	.14	6.8	20	68	A. H. 32 -49
5..	.059	.028	.214	.090	.023	.65	3.62	.48	.13	9.5	20	79	A. H. 25½ -8
6..	.057	.033	.263	.197	.033	.29	4.05	.58	.13	12.0	21	76	A. H. 56 -6
7..	.058	.034	.275	.058	.026	1.01	4.80	.59	.13	12.8	23	85	A. H. 46 -6
8..	.069	.031	.212	.075	.025	.92	3.10	.46	.15	11.0	9	59	A. H. 32 -28
9..	.066	.032	.234	.063	.026	1.04	3.58	.49	.14	10.2	21	89	A. H. 32 -43
10..	.068	.028	.244	.054	.022	1.25	3.60	.42	.12	11.4	21	89	A. H. 32 -54
11..	.066	.029	.249	.077	.029	.66	3.80	.59	.16	13.2	21	89	A. H. 80 -4
12..	.069	.045	.295	.045	.024	1.54	4.26	.65	.15	15.1	23	81	A. H. 45 -8
13..	.070	.032	.244	.077	.024	.91	3.52	.47	.13	9.8	23	93	A. H. 32 -7
14..	.066	.032	.236	.027	.021	2.40	3.55	.49	.18	11.0	23	91	A. H. 32 -14
15..	.064	.029	.226	.075	.023	.86	3.54	.45	.13	8.3	21	90	A. H. 17 -1
16..	.067	.029	.258	.053	.026	1.26	3.85	.43	.11	12.0	21	86	A. H. 56 -4
17..	.069	.023	.280	.106	.041	.41	4.10	.34	.08	13.7	21	68	A. H. 56 -1
18..	.062	.031	.275	.075	.030	.81	4.47	.50	.11	11.8	21	76	A. H. 46 -5
19..	.068	.036	.265	.056	.025	.81	3.90	.53	.14	11.3	21	76	A. H. 18 -4
20..	.070	.026	.316	.089	.032	.67	4.55	.37	.08	12.0	21	83	A. H. 54 -1
21..	.072	.029	.243	.056	.024	.84	3.36	.38	.12	9.6	21	94	A. H. 32 -44
22..	.074	.031	.242	.066	.022	1.11	3.28	.43	.13	13.6	21	86	A. H. 80 -3
23..	.079	.030	.228	.032	.019	2.43	2.88	.58	.13	8.2	21	89	A. H. 25½ -6
24..	.078	.029	.221	.041	.014	1.85	2.91	.38	.13	9.7	21	94	A. H. 17 -3
25..	.071	.043	.286	.244	.038	.29	4.04	.60	.15	14.9	21	70	a A. H. 56 -7
26..	.071	.037	.282	.122	.034	.58	3.06	.52	.13	13.8	21	56	A. H. 56 -2
27..	.071	.033	.278	.097	.038	.71	3.82	.47	.12	13.8	23	67	A. H. 46 -4
28..	.078	.033	.282	.138	.033	.57	3.03	.42	.12	11.5	19	80	A. H. A3 -3
29..	.083	.039	.202	.029	.032	.84	2.44	.47	.19	13.3	9	72	A. H. 32 -21
30..	.081	.028	.197	.028	.030	2.92	2.44	.35	.14	7.0	21	77	A. H. 32 -51
31..	.082	.043	.247	.098	.033	.82	3.00	.53	.18	.....	21	85	A. H. 74 -3
32..	.089	.028	.190	.038	.021	3.24	2.12	.32	.15	6.9	21	82	A. H. 32 -50
33..	.082	.043	.249	.098	.033	.82	2.92	.53	.18	.....	21	46	A. H. 74 -2
34..	.089	.027	.274	.033	.024	2.72	3.04	.30	.10	10.8	21	86	A. H. 80 -1
35..	.080	.036	.272	.033	.024	1.08	3.06	.40	.13	7.4	21	59	b A. H. 56 -7
36..	.081	.041	.265	.084	.028	.97	3.26	.50	.15	12.9	21	85	A. H. 53 -2
37..	.092	.028	.239	.018	.021	5.00	2.60	.30	.12	9.8	21	82	A. H. 25½ -4
38..	.094	.028	.199	.033	.019	2.80	2.03	.30	.15	8.4	21	79	A. H. 56 -7
39..	.097	.043	.315	.074	.038	1.31	3.25	.45	.14	14.2	21	82	A. H. 45 -10
40..	.103	.033	.238	.040	.023	2.10	2.30	.32	.14	8.6	21	87	A. H. 18 -3
41..	.117	.046	.230	.087	.030	1.16	1.97	.40	.20	13.0	23	84	A. H. 45 -9
42..	.127	.036	.258	.048	.019	2.75	3.04	.28	.14	7.8	20	88	A. H. 32 -52

a Upper.

b Lower.

TABLE IV.—Analyses of California lemon-grove soils, grouped according to percentage of mottled leaves, each group including eight groves

Group.	Percentage of—					Ratio of—				Moisture equivalent.	Mottled leaves.
	Humus.	Total nitrogen.	Organic carbon.	Mineral carbonates.	Mineral bicarbonates.	Humus to lime.	Carbon to humus.	Nitrogen to humus.	Nitrogen to carbon.		
1.....	0.066	0.036	0.241	0.062	0.023	1.06	2.74	0.545	0.149	13.1	92
2.....	.081	.033	.258	.050	.024	1.04	3.18	.407	.128	10.6	87
3.....	.087	.037	.265	.073	.029	1.19	3.05	.425	.139	11.5	82
4.....	.072	.033	.237	.097	.029	.74	3.29	.425	.139	11.0	76
5.....	.070	.033	.253	.089	.029	.79	3.01	.471	.126	11.3	58

RELATION OF HUMUS IN THE SOIL TO MOTTLING OF THE LEMON LEAVES

It was found that the humus content of the soil in the orange groves varied inversely with the leaf mottling. In the case of the lemon groves no definite relation appears at first sight to exist between these two factors. However, a comparison of Table II with Table IV shows that the humus content of most of the orange groves was much higher than that of the lemon groves. It will be noted from Table IV that the humus content of the lemon-grove groups was in every case less than one-tenth of 1 per cent, an extremely low value. Orange groves in which the humus content approximates 0.1 per cent (Table II) show as high a percentage of mottling as the lemon groves. It would therefore appear that the humus content in the lemon groves is less than is necessary for the growth of a leaf comparatively free from mottling, assuming that lemon leaves would mottle to the same extent as orange leaves under the same conditions.

RELATION OF MINERAL CARBONATES IN THE SOIL TO LEAF MOTTLING

An indication of a slight relationship between the mineral carbonate content of the soil and the percentage of mottled leaves was obtained in the case of the lemon groves, as shown in figure 4. The correlation coefficient is  $-0.31 + 0.09$ , the minus sign indicating that the mottling decreases as the mineral carbonates (chiefly lime) increase. This correlation coefficient would indicate an association between mottling and lime content of about 10 per cent. The probable error is relatively so large that the result can be considered to be little more than indicative of the inverse character of the relationship. The percentage of mottling is very high even in the case of the highest lime content. The average amount of lime carbonate in the lemon-grove soils was about the same as in the orange-grove soils.

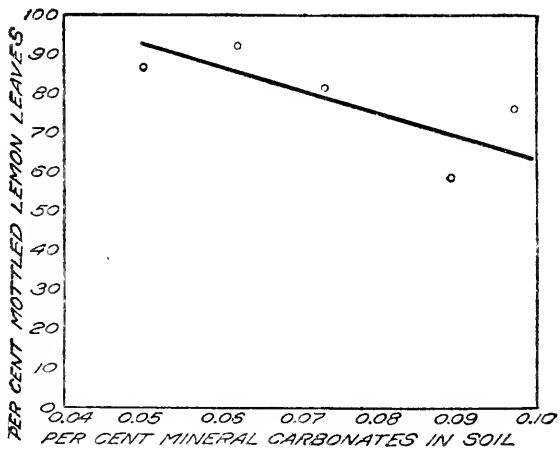


FIG. 4.—Graphical presentation of the relationship between the mineral carbonates in the soil and the percentage of mottled lemon leaves (from data in Table IV).

It is recognized that the lime-carbonate content is very low in all the soils represented in these areas and possibly a higher range of this constituent would bring out more definite results. The data presented are not sufficient to justify recommending the application of lime to lemon

groves which are low in this constituent, but lime experiments with the lemon would appear to be more promising than with the orange, other conditions being the same.

No correlation was found to exist between organic carbon or total nitrogen and mottling, the correlation coefficient in each case being no greater than the probable error.

#### SUMMARY

Mottle-leaf of *Citrus* trees is characterized by the disappearance of chlorophyll from parts of the leaf, the portions farthest removed from the midrib and larger veins being first affected. As the disturbance progresses, the yellowish spots increase in size until the only remaining chlorophyll is confined to narrow areas along the midrib and the larger veins. The advanced stages are accompanied by a marked decrease in the size, quality, and yield of fruit. No organism has yet been proved to be causally associated with mottle-leaf, but the *Citrus-root* nematode has been found by Thomas to be widely distributed in mottled districts.

Mottle-leaf is found in most *Citrus*-fruit sections of California, but is more prevalent in some districts than in others. All the *Citrus* fruits grown in California are affected, including the Washington Navel, Thompson Improved Navel, and Valencia orange, grapefruit, tangerine, and lemon.

The conclusions of the present paper are based upon a field and laboratory study of 130 orange groves and 45 lemon groves, located mainly in Riverside and San Bernardino Counties, Cal. The percentage of mottled leaves was determined by examining 10 to 12 typical trees in each grove. A soil sample 3 feet in depth was taken near each tree, each foot sample being kept separately. These samples were analyzed for humus, organic carbon, mineral carbonates, bicarbonates, and total nitrogen.

During the earlier stages of mottling no serious reduction in yield was observed. The fruit yield was apparently not seriously reduced on either orange or lemon trees which had about 20 per cent of their leaves mottled. Sour-orange stock was found to induce more severe mottling in orange trees than sweet-orange stock, other conditions being the same. A mixed grove of Washington Navel and Valencia oranges showed no difference in the amount of mottling of these two varieties.

Badly mottled orange trees cut back and rebudded on the stumps produce badly mottled new top growth; and unless the soil treatment of such groves is changed, the mottling persists.

There was no noticeable difference in the amount of leaf mottling in groves on different soil types, other conditions being the same.

Orchards fertilized with organic substances, such as stable manure or cover crops plowed under, usually showed less mottling than groves supplied principally with commercial fertilizers. Groves which for some years had received only the "complete" fertilizers in general use in the

areas studied were badly mottled in all cases, so far as observed in these studies. This was also the case where sodium nitrate was used alone or as the principal fertilizer for some years.

The results of the soil analyses show in the case of oranges a marked inverse correlation between the humus content of the soil and the percentage of mottling, the latter tending to diminish as the humus content increases. An impartial statistical study of the data from the individual orange groves shows that approximately one-half the mottling can be accounted for by the low humus content of the soil.

The humus content of the lemon soils studied is much below that of most of the orange soils, averaging less than 0.1 per cent. This amount of humus is apparently too low to produce a normal foliage growth, all of the lemon groves being badly mottled.

No correlation was found between the mineral carbonates of the soil and the mottling of orange trees. In lemons the mottling decreased slightly as the mineral carbonates increased, but the correlation is low. The lime content of nearly all the Citrus soils studied is low, and the effect of heavy applications of lime can only be determined by suitably controlled field experiments. The present study indicates that the application of lime would be more likely to benefit lemon trees than orange trees.

The percentage of mottled leaves on orange trees is definitely correlated with the increase of the ratio of organic carbon to humus, indicating the importance of the organic matter in the soil being well decomposed.

No relation was found between the percentage of leaves mottled and the total nitrogen content in the soil in either the orange groves or the lemon groves studied.

The principal conclusion of this investigation is that the mottling of orange trees in the areas studied is definitely correlated with the low humus content of the soil, the mottling diminishing as the humus content increases. A study of the data by statistical methods shows that approximately one-half of the mottling can be accounted for on this basis. The incorporation of organic matter with the soil in such a manner as to be accessible to the roots during its decomposition is indicated as a promising treatment for mottle-leaf.

PLATE H

Various stages in mottle-leaf of the orange.

(740)



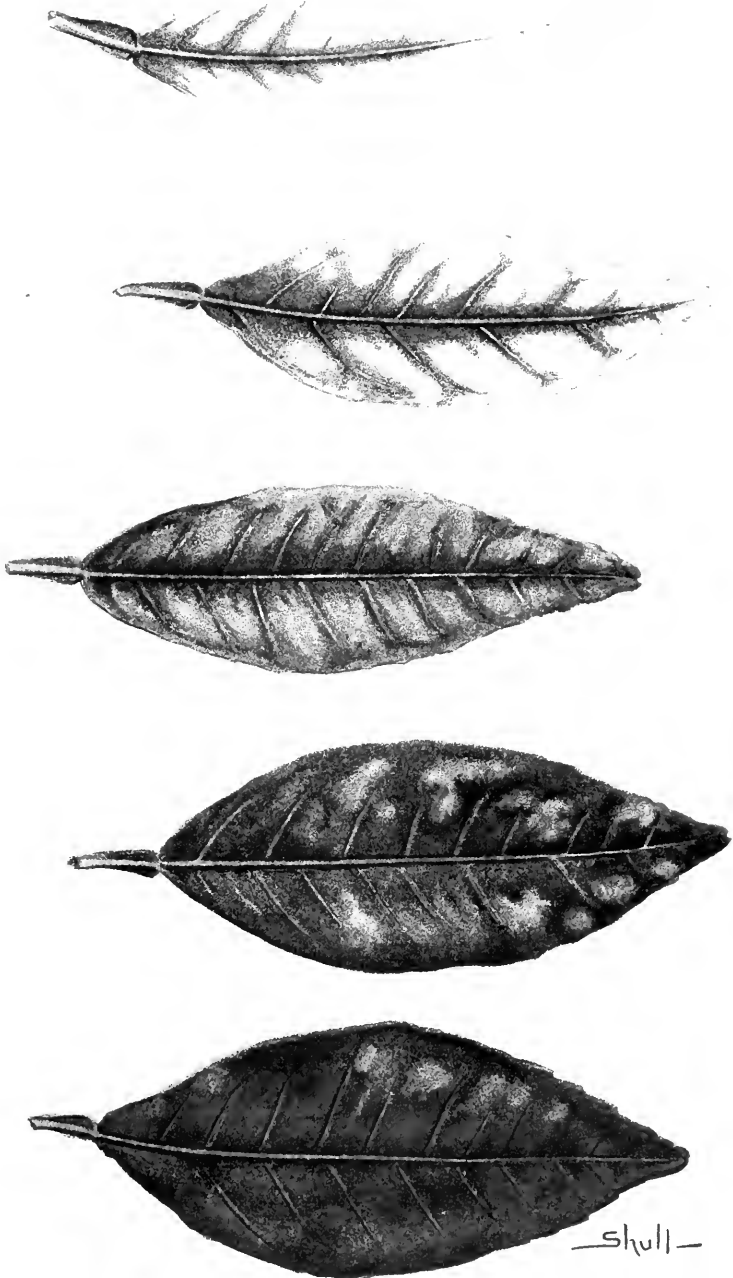






PLATE XCVI

Orange leaves showing mottle-leaf.





PLATE XCVII

A more advanced stage of mottle-leaf of orange, showing the reduction in the size of the leaves.





# VEGETATIVE SUCCESSION UNDER IRRIGATION

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## INTRODUCTION

The data given in the following pages are compiled from observations made during the growing seasons of 1912, 1913, and 1914, on a ranch situated near Rock River, Albany County, Wyo., in the southeastern part of the State. The Union Pacific Railroad makes the altitude at the station 2,105 meters. As the part of the ranch with which we are concerned lies about 3 miles up Rock Creek, the altitude will approximate 2,134 meters, a figure that corresponds very well with the general elevation of the Laramie Plains (including the Laramie Basin), of which this is a section.

Several years ago the hay yield of the ranch, obtained entirely from the natural meadow lands, fell short of the consumption. Means were taken to increase the hay acreage by the transformation of bench land to meadow, a transformation accomplished simply by flooding during the successive growing seasons. This method has proved so successful that each succeeding year has seen greater and greater areas flooded until now large reservoirs (Pl. CI, fig. 2) are required to augment the water supply during the midsummer season.

Of course it takes several seasons to complete this change, and the following notes are the result of a study of the various phases of vegetation through which the bench land passes in this transition to meadow. At the time these observations were made (1913 and 1914) several adjoining tracts, each flooded for the first time in a different season, furnished an unusual opportunity for the comparison and study of their development from year to year and at the same time gave striking physical evidence of actual differences in the vegetation present. I refer to the varying tones of green which the different tracts assumed as the season advanced—a condition so marked that with a very little practice one could ride over the ranch and state authoritatively that this section has been under water for four years, this for two, and so on. Indeed, it was this beautiful blotching of the landscape with various shades of green and brown that led to the discovery of what was really happening.

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<sup>1</sup> This work was done under the direction of Dr. Aven Nelson, of the Wyoming Experiment Station. If the conclusions, based on a study that was conceived in a spirit of helpfulness, prove of value, the credit can not be too largely assigned to the active interest and kindly encouragement of my adviser. The distressing difficulties under which the photographs were secured could not have been overcome except by Dr. Nelson's perseverance and constant help. I wish to thank Prof. A. S. Hitchcock, of the United States Department of Agriculture, for determining my material of the genus *Agropyron*; also Mr. V. H. Rowland, who furnished the determinations of the difficult genus *Carex*.

A collection of plants illustrating the salient features of the Rock Creek upland and meadow floras and substantiating the points brought out in this study is deposited in the Rocky Mountain Herbarium at Laramie, Wyo.

## GEOLOGY OF ROCK CREEK REGION

The geological formation<sup>1</sup> belongs to the lower part of the Montana group of the Cretaceous, characterized by sandstones and carbonaceous shales with local coal deposits. The depth of this deposit is said to average over 305 meters, and there is no evidence that Rock Creek has more than scratched the surface, so the soil of the region is of nearly uniform character. Sandstones beneath the coal contain various fossils of particular interest, because about 50 per cent belong to genera represented there to-day, as cottonwood (*Populus* spp.), alder (*Alnus* spp.), birch (*Betula* spp.), willow (*Salix* spp.), and others. The rather soft surface shales and sandstones are occasionally exposed, but more frequently are covered with a fine gravel wash, somewhat disguised by reason of the growth of short grasses and xerophytic shrubs. These remarks, of course, apply only to the land above the stream. Rock Creek, like all the streams in the Laramie Basin, flows through a valley varying in width and filled with alluvial deposits, in many places 50 to 60 feet deep. The character of the upland soils is also typical of a great deal of the Laramie Basin, so that from an agricultural standpoint the results of this study are applicable to a much larger region than that in which the actual observations were made.

## CLIMATE OF ROCK CREEK REGION

The climate of Rock Creek is essentially that of the Laramie Basin. Meteorological records kept at the State University at Laramie since 1891 show an average rainfall of about 10 inches and a mean annual temperature of about 40° F. The monthly averages for 15 years are given in Tables I and II.

TABLE I.—*Monthly means of precipitation (in inches) at Laramie, Wyo., 1891-1905*

January.....	0.23	May.....	1.47	September.....	0.92
February.....	.34	June.....	1.24	October.....	.79
March.....	.83	July.....	1.40	November.....	.22
April.....	1.14	August.....	.99	December.....	.33

TABLE II.—*Monthly means of temperature (°F.) at Laramie, Wyo., 1891-1905*

January.....	21.6	May.....	47.4	September.....	51.8
February.....	20.3	June.....	56.6	October.....	42.1
March.....	28.4	July.....	62.3	November.....	31.1
April.....	37.3	August.....	61.9	December.....	21.8

The higher temperatures are of short duration, and the maximum rarely reaches 90° F. All the nights are cool. The flora is thus, of necessity, composed of plants that have become adapted not only to the low average temperature and the aridity of the plains but also to the

<sup>1</sup> Darton, N. H., and Siebenthal, C. E. Geology and mineral resources of the Laramie Basin, Wyo. A preliminary report. U. S. Geol. Survey Bul. 364, 81 p., 2 pl. 1905.

short season; for although September often draws to a close before the first killing frost, the next June may be half gone before the cottonwoods along the streams have flaunted anything like full-grown leaves. Of course, grasses and bulbous and thick-rooted perennials have beautified the plains with flower or verdure long before the last frost is out of the meadow lands, or at least before the spring freshets have subsided enough to permit the growth of the meadow plants.

#### PHYSIOGRAPHY OF ROCK CREEK REGION

Physiographically the Rock Creek ranch is divisible into four regions or units which for convenience may be designated and defined as follows: (1) The stream valley (Pl. XCVIII), practically synonymous to the flood plain of the creek and characterized by the natural meadows, willow thickets, swamps, and cottonwood timber; (2) the bench slope (Pl. XCIX; C, fig. 1), representing the sides of the stream valley; (3) the draws, or gullies (Pl. C, fig. 2), occurring on the bench proper and breaking through the bench slope at intervals; and (4) the bench land (Pl. CI, fig. 1), flat, short-grass, upland plains.

A knowledge is needed, of course, of the original flora of the flooded lands, in order to comprehend the changes in vegetation which are going to take place in some of the regions as the result of irrigation. Accordingly the immediately succeeding paragraphs are devoted to a description and to an analysis of this flora.

#### PHYTOGEOGRAPHY OF ROCK CREEK REGION

The lists of plants under "Phytogeography of Rock Creek region" represent only those that contribute a present or later value toward the working out of the problem in hand. For additional species noted, mostly of interest only to the botanist, the reader is referred to the lists of minor plants on pages 757-758. In some instances plants here referred to under their generic name are given their specific designations later and in these instances will not be found in the supplementary lists.

#### BENCH FLORA

##### CHARACTERISTIC BENCH-LAND PLANTS

<i>Agropyron</i> spp. (four species).	<i>Oxytropis monticola</i> Gray.
<i>Buchloe dactyloides</i> (Nutt.) Engelm.	<i>Pentstemon angustifolius</i> Pursh.
<i>Oryzopsis hymenoides</i> (R. and S.) Ricker.	<i>Chrysothamnus frigidus</i> Greene.
<i>Eurotia lanata</i> (Pursh) Moq.	<i>Artemisia frigida</i> Willd.
<i>Potentilla effusa</i> Dougl.	<i>Lygodesmia juncea</i> Don.
<i>Astragalus</i> spp. (five species).	<i>Tetradymia inermis</i> Nutt.

The ecologist will note plants in this list representative of well-known plant associations, such as the short-grass and the wheat-grass; the purpose of these lists, however, is not to classify the plants of a given physiographic unit but rather to treat such plants as a complex the limits of which are

determined purely by the bounds of the unit, as indicated above. Since these bounds are of more or less arbitrary definition in themselves, the disposition of plants within them is of like nature, though it may be said that, in general, the fullest development of a given species normally occurs within the unit in which it is placed.

Any one of the four wheat-grasses may be dominant or principal species—that is, it may make up the bulk of the vegetation over large areas of this unit (Pl. CII, fig. 2). The kind with which we are most concerned, however, reaches its best development in another complex.

Indian millet (*Oryzopsis hymenoides*), though of great importance from a nutritive standpoint,<sup>1</sup> is largely confined to loose, somewhat sandy soils; and therefore its possibilities for forage development are limited to regions of that nature.

Attention is called to the large number of legumes in this complex—five kinds. These will be augmented by five more, distributed among the remaining complexes. Legumes require rich soils of high lime content, the significance of which will develop later.

The remaining plants are nearly all xerophytes, species eminently fitted in one way or another to withstand the rigors of the environment in which they live. It is not surprising that such plants will take no part in an artificial transformation of this bench to meadow, where new conditions are suddenly introduced which are directly opposed to those to which these species have become so well adapted. Their disappearance in many cases means the loss of the most nutritious plants on the range.

#### CHARACTERISTIC DRAW, OR GULLY, PLANTS

*Bouteloua oligostachya* (Nutt.) Torr.  
*Koeleria cristata* (L.) Pers.  
*Stipa comata* Trin. and Rupr.  
*Carex* spp. (two species).  
*Zygodenus intermedius* Rydb.  
*Delphinium Geyeri* Greene.

*Astragalus* spp. (two species).  
*Lupinus parviflorus* Nutt.  
*Antennaria parvifolia* Nutt.  
*Artemisia cana* Pursh.  
*Grindelia subalpina* Greene.

The bench lands are interrupted at irregular intervals by swales, or draws. These carry away the surplus surface water to the stream, so that those that drain a considerable area become gulches. Plate C, figure 2, gives a good average idea of this topographical feature. The chief difference, as compared to the bench proper, is the more constant water supply, which is somewhat greater and which lasts longer. It is not surprising, therefore, that over 69 per cent of its more characteristic plants (as given above) will persist (in some cases attain greater development) during the first season under irrigation.

Among those that will not be able to stand the new conditions are *Delphinium Geyeri* and *Zygodenus intermedius*, both a constant menace to

<sup>1</sup> Knight, H. G., Hepner, F. E., and Nelson, Aven. Wyoming forage plants and their chemical composition—Studies no. 3. Wyo. Agr. Exp. Sta. Bul. 76, 119 p., 50 fig. 1908.

stock because of poisons they contain. The latter is peculiar in its habitat relations. It appears in drier parts of meadows that have been established for several years, but does not seem to stand the sudden change to meadow conditions. This may be explained by the fact that it grows in the lower parts of the draw, where the change in moisture content becomes greatest. When it later invades the meadow, places frequently exist that more nearly conform to its usual habitat. At any rate, the new meadows are free from it for some time.

#### CHARACTERISTIC BENCH-SLOPE PLANTS

*Agropyron Smithii* Rydb.  
*Elymus condensatus* Prsl.  
*Delphinium Menziesii* DC.  
*Arabis hirsuta* Scop.

*Potentilla pennsylvanica* L., var. *strigosa*  
 Pursh.  
*Astragalus* spp. (three species).  
*Solidago concinna* A. Nels.

The change from upland to lowland is very abrupt, as shown in Plates XCI and C, figure 1. The conditions are in many respects similar to those of the preceding region, except that the water supply is greater and even more constant. This permits the growth of various mesophytic herbs and shrubs (for lists of these see pp. 757-758.) The upper part of the slope is more or less like the bench, depending upon the local variation in the abruptness of the incline. Therefore the bench-slope plants can not be satisfactorily segregated from those of other units, since species characteristic to them find within the borders of the bench slope entirely suitable habitats.

*Agropyron Smithii* prefers a more constant water supply than its relatives, so it is placed here, though a complete list of the plants of either of the complexes previously discussed would contain it. *Elymus condensatus* likes a moist, sunny, well-drained situation; consequently it is out of the question as a meadow plant and is not of much value under any condition, being coarse and woody. *Delphinium Menziesii* no doubt is poisonous, but its numbers are ordinarily so limited that its interest lies chiefly in the fact that it is a relative of *Delphinium Geyeri*. *Arabis hirsuta* at times acts very much like a weed. One of the three vetches at home here is *Astragalus tenellus*, the only upland vetch in the new-meadow development.

#### METHOD OF IRRIGATION USED

Before considering the transition itself—that is, the artificial transformation of upland to meadowland—the reader should know something of the mechanical means employed. Although the ranch owners have taken some pains to build large reservoirs, such as the one shown in Plate CI, figure 2, the actual distribution of the water over the land is accomplished in the crudest way imaginable. A ditch is built along what is obviously the highest ridge of a given area and provided with the

customary boxes where they seem to be needed. Very few laterals are used, and there is no secondary ditching. The ditch is opened at intervals and the water allowed to creep out over the land. Naturally it follows the path of least resistance. In order that it may reach some of the high places as well as all of the low, temporary ridges of earth are thrown up, which may perhaps be termed "dikelets," since they are too small and unstable to be called "dikes," but which serve the same purpose. Although, of course, this is irrigation in a broad sense, "controlled flooding" would be a term both more accurate and more appropriate for a method in which science plays so small a part. The remarkable thing about this is that the area so treated really receives a very even soaking; and if there are a few higher places that are not as wet as the land as a whole, so there are higher places in the natural meadow that are always relatively dry. After all, then, controlled flooding furnishes conditions almost analogous to those present in the natural meadow, an important point which, once attained, makes it possible to realize that these uplands, geologically the same as the valley they inclose, potentially are capable of the same vegetative results.

#### THE TRANSITION

The first interest in this transition may be expressed by the question, "What happens to the upland plants?" The great bulk of them perish very soon. However, assuming that the water is turned on in the spring and allowed to remain until the soil is saturated and is thereafter replaced at intervals frequently enough to keep the ground wet (the common procedure), many of the plants may reach maturity. These readily fall into two classes: First, those of little economic value, either because of actual numerical or structural deficiency or because of lack of ability to cope successfully with the new conditions; and, second, those which flourish under the new conditions and often possess great economic value. The following represent the first class:

#### UPLAND PLANTS OF SOME IMPORTANCE FIRST SEASON IRRIGATED

<i>Oryzopsis hymenoides</i> (R. and S.) Ricker.	<i>Pentstemon exilifolius</i> A. Nels.
<i>Stipa comata</i> Trin. and Rupr.	<i>Chrysothamnus frigidus</i> Greene.
<i>Potentilla pennsylvanica</i> L., var. <i>strigosa</i>	<i>Gaillardia aristata</i> Pursh.
Pursh.	<i>Solidago concinna</i> A. Nels.
<i>Astragalus tenellus</i> Pursh.	<i>Tetradymia inermis</i> Nutt.
<i>Lupinus parviflorus</i> Nutt.	

I have already spoken of the limitations of Indian millet. Not infrequently though, an area of upland will contain one to several spots, which with controlled irrigation could be much more profitably used for growing millet than rushes. The rushes are not nearly so nutritious, but, as will be shown later, will monopolize these sandy places under the controlled-

flooding system. With moderate moisture millet becomes even prolific and attains a height of about 60 cm.

*Stipa comata* is a good pasture grass, but the long awns may be fatal to stock because of the presence of tiny barbs. On the range the animals seek it either before the awns have developed or after they have fallen. Altogether, it is fortunate that it is able to survive only one season of meadow conditions.

The next plant that needs more than mention is vetch, the only one persisting of the 10 upland kinds listed. Its ability to persist makes it the connecting link between the upland vetches and those of the lowland which are to invade the developing meadow.

The lupins (Pl. C, fig. 2; CII, fig. 1), which persist a summer under controlled flooding, could probably be perpetuated indefinitely under controlled irrigation. However, since some species are strongly suspected of being poisonous to stock, at least during certain periods of their growth, their cultivation can not be advised, unless careful means are taken to ascertain this fact as regards the local species. These are numerous, and some are known to be not only harmless but of great value. Such is the case with an Idaho species which I have seen form, with the farmer's encouragement, a nearly pure stand on rather sandy bottom lands. This was cut just before the pods became dry, and when fed mixed with hay, both cattle and horses relished and thrived on it during the winter.

The next list represents the chief contribution of the upland flora to the growing meadow formation.

#### UPLAND PLANTS TENDING TO DOMINANCE THE FIRST SEASON IRRIGATED

<i>Agropyron albicans</i> R. and S.	<i>Carex siccata</i> Dewey.
<i>Agropyron dasystachyum</i> (Hook) Scribn.	<i>Carex stenophylla</i> Wahl.
<i>Agropyron molle</i> Rydb.	<i>Arabis hirsuta</i> Scop.
<i>Bouteloua oligostachya</i> (Nutt.) Torr.	<i>Antennaria parvifolia</i> Nutt.
<i>Koeleria cristata</i> (L.) Pers.	<i>Grindelia subalpina</i> Greene.

Nearly a dozen upland plants not only persist under controlled flooding but even flourish, at least when they happen to be established on a relatively high spot. With the advent of the first meadow plants (some of which are next listed) two opposing elements must be considered, one of which has had to adapt itself to an environment partially new.

#### MEADOW PLANTS APPEARING ON UPLAND THE FIRST SEASON IRRIGATED

<i>Hordcum jubatum</i> L.	<i>Astragalus hypoglottis</i> L.
<i>Sporobolus brevifolius</i> (Nutt.) Scribn.	<i>Oxytropis deflexus</i> DC.
<i>Juncus longistylis</i> Torr.	<i>Plantago eriopoda</i> Torr.
<i>Astragalus Bodinii</i> Sheld.	

By the end of the first summer these typically meadow plants are almost certain to be represented, sometimes only by scattered individ-

uals, again completely appropriating areas left barren because of the death of upland plants that were unable to grow in the new environment.

These two elements furnish the foundation for two distinct lines of development which the upland may undergo in its transformation. The value of the respective components may be noted profitably now.

*Agropyron Smithii*, strangely enough, seems to be the only species that develops either vigorously or abundantly. There are at least two possible reasons for this. Attention has already been called to the fact that it prefers the slopes where the moisture content is greater and more uniform, so it may be the only species that is capable of using to advantage the increase in available moisture. Or it is not impossible that some of the other kinds, under the new conditions, become indistinguishable from the true *A. Smithii*, with which they not infrequently grow and from which they are separated by such characters as pubescent glumes and comparative awn development, characters which there is good reason to believe are easily modified by environmental factors.

*Koeleria cristata* and *Bouteloua oligostachya* and the two sedges noted are valuable, but the controlled-flooding method of irrigation caused their almost total disappearance by the second season.

*Arabis hirsuta* develops best, as will be evident when controlled irrigation is used, becoming one of the few native weeds.

*Antennaria parvifolia* is an everlasting nuisance on comparatively dry places. Plate CII, figure 1, shows its excessive development along a meadow edge. It readily succumbs to too much water, but unhappily the regular order of succession seems to mean that it will usually be replaced by *Pedicularis crenulata*, the weed that so frequently ruins meadows.

*Grindelia subalpina* reaches its greatest development the second season, as a weed.

*Hordeum jubatum* is not necessarily a meadow weed by any means, but since it occurs as such in this region it is included in this list of meadow plants. It is sometimes fed, but is dangerous because of the barbed awns. Like wheat-grass, interest in it has just begun.

*Sporobolus brevifolius* and *Plantago eriopoda* are of saline or subsaline habitat, and therefore their presence is significant. When an alkaline place appears, the invasion of this valuable<sup>1</sup> grass is the best thing that can happen; if it succeeds in establishing itself, it uses a spot that would be occupied by the plantain or later by one or both species of *Sagittaria*. (See "Minor plants," pp. 757-758.)

*Juncus longistylis* is destined to play an important part in the meadow development. Its nutritive value is rightly regarded as good, but it suffers by comparison of its analysis with that of wheat-grass.

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<sup>1</sup> Knight, H. G., Hegner, F. E., and Nelson, Aven. Op. cit.



It is not surprising to find these meadow vetches appearing so early when the numerous upland species are recalled, a proof of the upland and lowland similarity as regards the soil and a further index to its character as pointed out in a preceding paragraph. *Astragalus bodinii* develops long, spreading stems which make it difficult to harvest, but its high nutritive value and the evident relish with which it is eaten overcome this drawback. Though its development may be arrested, it will ultimately form a large part of the hay crop.

The other species, *A. hypoglottis*, does not seem able to hold its own long where *A. Bodinii* is present, and the latter will eventually largely replace it.

*Oxytropis deflexus* belongs to a different habitat and is to be regarded here as a stray. It may be poisonous, but it rarely occurs in any abundance.

Such, then, is the condition of vegetation at the end of the first season. During the second season one of two possible lines of development become definitely established. Either the forming meadow enters upon what may be called the *Agropyron* phase or else the *Hordeum* phase. As to which phase appears depends on the following condition: It was observed that the ranch company sometimes ran short of water; and in such a case the newer areas were kept only moist, not wet like those regions longer established, for which the greater part of the available water was conserved. Granted that one or more of the wheat-grasses happened to be dominant over a given area the year before (as was very likely the case) a practically pure stand of this grass was the result (Pl. CV). The presence of an occasional tuft of *Hordeum jubatum* should be noted.

Before tracing through the development of this phase, the other possibility should be mentioned. As a curtailed water supply was essential to the dominance of *Agropyron* spp. at this time, so an abundance produced the *Hordeum* phase (Pl. CIII, figure 1). This phase is more strongly defined than the other because *Hordeum jubatum* is always the dominant plant. Its chief competitor is *Grindelia subalpina*, which is just as worthless and just as truly a weed.

Here is striking evidence of the aggressive character of a plant which because of its uselessness we call a "weed" and of the unobtrusive character of an indigenous and valuable grass. That is, the development of the *Agropyron* association is primarily dependent on its local dominance the preceding year. When such is not the case, the artificial condition described above tends to produce a mixed association characterized by one or all of those plants which largely composed the upland and the meadow elements, as discussed previously. Chief among these will be *Bouletoua oligostachya* and *Koeleria cristata*, the two sedges *Carex siccata* and *C. stenophylla*, *Antennaria parvifolia*, the meadow vetches

*Astragalus Bondinii* and *A. hypoglottis*, and *Juncus longistylus*. *Grindelia subalpina* and *Hordeum jubatum* are usually present in varying abundance but never attain dominance. This development should be contrasted with that of the *Hordeum* association. Here we have dependence only on the water factor whereas the *Hordeum* association was dependent not only on this but also primarily on its own dominance the preceding year. In the case of an abundant water supply, whether or not *Hordeum jubatum* was locally present, let alone dominant, the first season, it attained dominance the second. Attention has also been called to the stray plants of *H. jubatum* appearing either in the pure or mixed types of the *Agropyron* association, a further indication of its aggressiveness.

In the outline of the meadow activities during the second season no mention has been made of possible new emigrants from the lowlands. As some are destined to have a most important share in the further history of the evolutive upland, the following list is given:

MEADOW PLANTS APPEARING ON UPLAND SECOND SEASON IRRIGATED

*Deschampsia caespitosa* (L.) Beauv.  
*Juncus balticus* Willd. var.  
*Juncus bufonius* L.  
*Rumex mexicanus* Meisn.  
*Epilobium Drummondii* Haussk.

*Glaux maritima* L.  
*Orthocarpus luteus* Nutt.  
*Veronica peregrina* L.  
*Gnaphalium palustre* Nutt.  
*Rudbeckia hirta* L.

*Deschampsia caespitosa* is one of the best meadow grasses. It is frequently associated with *Calamagrostis canadensis* (Michx.) Beauv., which, strangely enough, seemed to be completely absent in the Rock River territory. It is highly probable, however, that it would bear the same relation as *D. caespitosa* toward the various factors involved in the present problem. It is highly nutritious,<sup>1</sup> material collected at this altitude containing 7.76 per cent of crude protein (water-free).

*Juncus balticus* ranks with *J. longistylus* in forage value. *J. bufonius* is a low diffuse annual.

*Rumex mexicanus* is always scattering in its distribution, but is usually a perceptible element in natural-meadow hay. It is probably to be regarded as a weed; it is coarse and certainly hard on sickle blades.

The rest are harmless herbs. *Orthocarpus luteus* under some conditions may become quite weedy in character. *Glaux maritima* furnishes by its presence another evidence of saline soil. It is as worthless for food as *Plantago criopoda*.

A careful examination of either of the associations defined above would show the presence of at least some of these plants. They would be scattered through the dominant species in an entirely inconspicuous manner. In a large way the *Agropyron* phase would contain a suggestion of *Deschampsia caespitosa*, *Juncus longistylus*, and *Orthocarpus luteus*,

<sup>1</sup> Knight, H. G., Hepner, F. E., and Nelson, Aven. Op. cit.

and other herbs, and the *Hordeum* phase, *J. balticus*, *Rumex mexicanus*, *Grindelia subalpina*, and others mentioned. However, this does not mean that *D. caespitosa*, for instance, would be absent from the latter phase, but merely that it would occur more frequently in the former; and so with *J. balticus*, which would be less abundant, though not necessarily lacking, in the *Agropyron* association, and so on.

Before the end of the second growing season seeds of the following meadow plants will have germinated and taken root in the upland, so that by the middle of the next (the third) summer, they will have become a factor to be reckoned with. Therefore their consideration at this time is not out of place.

MEADOW PLANTS APPEARING ON UPLAND THIRD SEASON IRRIGATED

*Carex nebraskensis* Dewey.  
*Carex Gayana* Desv.  
*Carex lanuginosa* Michx.

*Carex marcida* Boott.  
*Gratiola virginiana* L.  
*Agoseris glauca* (Pursh) Steud.

*Carex nebraskensis* is one of the commonest and most valuable<sup>1</sup> of our sedges. It grows in clumps and reaches a good height. It is very similar to *C. variabilis*, which is perhaps even more frequent.

*Carex Gayana* is often the principal species of a given area. It is much less robust than the other sedges and does not grow as tall. Another drawback is that it has a tendency to mature early and turn yellow, losing much in substance before harvest. *C. lanuginosa*, next to *C. nebraskensis*, is probably the most important of the sedges. It is highly nutritious,<sup>1</sup> and its long narrow leaves and slender stems make it quite grasslike.

The remaining species are mainly of interest because of their comparatively early appearance. They belong to that large series of meadow plants which are present here, absent there, and are usually late in coming in. A large proportion of the swamp species belong to this type (p. 753). By the end of the second summer, then, there has been a considerable invasion of meadow plants.

Plate CIII, figure 2, clearly shows what happens during the third season. The reader will recall the presence of rush in the second-year condition of the *Agropyron* association. Here it is rapidly replacing the wheat-grass (stunted by too much water) and in the fall this phase were better called the *Juncus-Carex*, or rush-sedge phase. The next summer (the fourth) it will ordinarily reach its highest development. "Ordinarily," because this step in the program is no doubt dependent on the resumption of the usual controlled flooding, a method which, it will be remembered, was temporarily forsaken because of insufficient water. During my observation this resumption always took place, because by this time the upland had reached the stage where a hay crop was assured and good treatment justifiable. Owing to the relatively dry conditions, *J. longi-*

<sup>1</sup> Knight, H. G., Hepner, F. E., and Nelson, Aven. Op. cit.

*stylus* was the more abundant rush rather than *J. balticus*, which likes the wetter areas; similarly *Carex Gayana* and *C. marcida* outranked *C. nebraskensis*. *C. lanuginosa* was not present. The hay made from this association for the fourth season showed a marked increase in the percentage of *Deschampsia caespitosa*.

In the *Hordeum* phase the evolution is somewhat different and often less rapid than in the rush-sedge phase. Controlled flooding, the factor which, by drowning all competition, made possible the dominance of *Hordeum jubatum*, the next year weakened this same dominance. *Juncus balticus*, and even *Deschampsia caespitosa*, are much better suited to prolonged wettings. The result was that by the fourth season, the rush-sedge phase had been evolved here also, but with less definiteness unless complete destruction of the virulent weed, *H. jubatum*, was hastened in a novel and unexpected way. In some cases this annihilation was actually accomplished by the sudden appearance of the smut, *Ustilago hordei* (Pers.) Kell. Acres of the grass were often affected, it being practically impossible to find a single plant which did not have all or nearly all of its heads completely smutted. The coming of the new enemy, coupled with the already serious crowding of plants better suited to the wet conditions, could result in only one thing. The rush-sedge supplanted the *Hordeum* phase, just as the latter had supplanted the *Agropyron* phase, except that in this case its components were other species. The rush was *J. balticus*, the sedges were *Carex nebraskensis* and *C. lanuginosa*, and the proportion of *D. caespitosa* was often much greater. When the smut did not appear, at least another year was necessary for the drowning out of *H. jubatum*.

Thus, by the fourth year, or at least by the fifth, the upland normally reached the condition we have called the "rush-sedge phase", a condition characterized by the presence of plants belonging in large part to the rush or sedge groups or families. This phase is more stable than either of the earlier stages (the *Agropyron* or *Hordeum*); but it, too, soon changes.

The growing abundance of *Deschampsia caespitosa* has been mentioned; in fact, in some cases it came on so rapidly that it became established at the same time as the *Juncus-Carex* phase forming what might be called the *Juncus-Carex-Deschampsia* phase. This was particularly likely to be the case when the upland passed through the *Hordeum* stage. At any rate, a year or two usually showed the condition illustrated in Plate CIV, figure 1. This appears to be a nearly pure stand of *D. caespitosa*; but in reality it contains a small percentage of rush, principally *J. balticus*, if its origin has been by way of the *Hordeum* phase, or of *J. longistylus* and sedge, if its development has been through the *Agropyron* phase.

At this time there is a tendency of certain plants to attain local dominance. Naturally among these are *Carex Gayana*, *C. nebraskensis*, and

*Astragalus Bodinii*. The species of *Carex* have a high water requirement and belong to the cycle resulting from the continuous use from the first of controlled flooding. The best development of the vetch was coincident to the *Agropyron* phase; otherwise its growth was limited to isolated, often more gravelly, elevated places of relative dryness. The exact conditions that produce these subphases, as they may be called, are not understood and represent only one of the many points which have yet to be fully worked out.

The *Deschampsia* phase is by far the most stable yet considered. Once established, controlled flooding seemed to satisfy its water requirement so exactly that its character fluctuated but little from year to year. The areas longest under water, however, gave evidence of two possible further changes; either a gradual increase in the abundance of rush (largely *Juncus balticus*) or a gradual increase in the number of kinds of plants. The former case meant the ushering in of the *Juncus balticus*, or wire-grass, phase the presence of which typifies swamp conditions and is the forerunner of the bog. Besides wire-grass, the hay harvest from this phase at Rock Creek contained some at least of the following plants:

SWAMP SPECIES TENDING TO INVADE WETTER PARTS OF MEADOW

*Calamagrostis hyperborea* Lange.  
*Glyceria borealis* (Nash) A. Nels.  
*Eleocharis palustris* (L.) R. and S.  
*Scirpus microcarpus* Presl.

*Habenaria viridiflora* Cham.  
*Rumex occidentalis* Wats.  
*Ranunculus reptans* L.

Of these plants only two are ordinarily of sufficient importance to deserve comment. *Eleocharis palustris*, naturally an inhabitant of pond margins, finds in the wire-grass phase an environment to which it is well suited and it soon becomes an important factor. Its value as forage is fully as great as that of the rushes.<sup>1</sup> *Scirpus microcarpus*, like the preceding, is indicative of marshy conditions. It is scattering in its distribution, but its presence is to be noted with satisfaction, as it is one of the few members of this phase that possess a considerable forage value.<sup>1</sup>

The other possibility, the gradual increase in the number of different species, is yet to be considered. This change is even slower than the other. For one thing, there is now a firmly established turf in which any plant finds it difficult to secure a foothold. Finally, however, now here, now there, some local variation in conditions or some factor which escapes our notice permits the invasion of many species, such as those listed under the heading "Meadow plants appearing at some later season" (p. 758). Only three of these are noteworthy: *Cicuta occidentalis* is a poisonous parsnip which for its best development needs the conditions that produce the wire-grass phase. *Pedicularis crenulata* is no doubt the worst weed of the natural meadows, but strangely enough does not seem to bother the made meadows until late. If it appears, the *Carex Gayana*

<sup>1</sup> Knight, H. C., Hepner, F. E., and Nelson, Aven. Op. cit.

subphase seems to be its most likely point of attack. *Carduus foliosus* is sometimes very troublesome. Fortunately it, too, is mostly confined to the natural meadow.

Gradually, then, the upland takes on the cosmopolitan character of the natural meadowland shown in Plate CIV, figure 2. In other words, those finer adjustments that involve plants which possess keener sensibility to moisture content and to slight soil variations are brought about by nature only after many years until finally there exists that marvelous complex of species growing in perfect equilibrium which is known as the natural meadow.

#### ECONOMIC APPLICATION OF OBSERVATIONS

Such, then, is the story of the artificial production of a natural meadowland. One distinct type of vegetation completely supplanted another type of vegetation. This change was relatively gradual and not abrupt; it was readily divisible into periods, each period or stage being characterized by certain species which dominated that period, only to be recessive in the next; and lastly, the forage values of the various plants have been given.

These observations have proved that it is possible to control the physical stages of the evolutive upland. Therefore, if stockmen desire to augment their yield of natural hay, their procedure should be as follows:

First, a careful study of the upland area to be transformed should be made. It was shown that in the case of the Rock Creek project the upland and lowland were of the same geological formation and the soils of essentially the same character. Presumably this is important, but no study has been made of dissimilar regions. The principal plants should be noted, and if the area were large those parts which supported a good stand of wheat-grass or lupin (see above) or Indian millet should be mapped out, because on this knowledge would depend an intelligent use of the available water.

Second, a surveyed and modern system of irrigation should be established for use on the tract, so that not only controlled flooding but also controlled irrigation could be employed as occasion demanded. The latter method is essential for the development of the *Agropyron* phase, which was eliminated at Rock River, unless by chance the water supply was curtailed and conditions produced simulating those made possible by controlled irrigation. Briggs and Shantz<sup>1</sup> have shown that *Agropyron Smithii* has a water requirement of 1,076 units for every unit of dry weight produced, as compared with alfalfa with 831. With facilities for controlled irrigation the farmer should be able to furnish with a little practice the amount of water required to develop to the fullest the

<sup>1</sup> Briggs, L. J., and Shantz, H. L. Relative water requirement of plants. *In* Jour. Agr. Research, v. 3, no. 1, p. 1-63, pl. 1-7. 1914. Literature cited, p. 62-63.

latent possibilities of this native grain. The advisability of the preservation of this phase from year to year is a little doubtful. It might be successful, but it would probably never give the yield to the acre that the *Deschampsia* phase furnishes and is less nutritious, unless the later phase contains a large percentage of *Juncus balticus*. Besides, it is not improbable that after a year or two *Hordeum jubatum* would be able to maintain itself in such abundance that it would ruin the wheat-grass crop (Pl. CII, fig. 2). Then, too, controlled irrigation is more expensive than controlled flooding, so that the question of practicability enters. Altogether, the evidence seems to indicate that, whenever possible, the *Agropyron* phase should be encouraged for a year, or possibly two, in order to eliminate the *Hordeum* phase (the constant result of controlled flooding) and then should be allowed to pass into the *Juncus-Carex* or *Juncus-Carex-Deschampsia* phase, according to the natural tendency.

In starting the transformation all precautions should be taken to eliminate the worthless *Hordeum* phase, a stage which, once established, means from one to several seasons lost, with no advantage to the farmer. In case wheat-grass was not present on the upland, controlled irrigation tended to the development of a mixed association.

Considering the additional expense, it is thought probable that in most cases the *Hordeum* phase had better be tolerated, as the transition may be more rapid to the *Deschampsia* phase.

The rush-sedge phase (the next, the reader will recall, in the cycle of normal succession) should not be encouraged. It is doubtful whether there is any escaping its presence, although a uniform and constant water supply is probably the chief factor that tends to modify it into the *Juncus-Carex-Deschampsia* phase. Care must be taken, lest with too much water it revert to *Juncus balticus*. Whether evolved from the *Agropyron* or the *Hordeum* phase, none of its chief components possess the nutritive value of *Deschampsia caespitosa*. *Carex lanuginosa* ranks the highest, but it is likely to form a subphase of its own.

The two principal species of the *Deschampsia* phase represent extremes in forage value. *D. caespitosa* is one of the most nutritious meadow plants<sup>1</sup> and *Juncus balticus* is one of the least nutritious. Hence, the importance of maintaining *D. caespitosa* as the dominant plant of this phase. This accomplished, the farmer has the best natural meadow the region affords, which will average 1½ tons to the acre and will remain for years free from meadow weeds.

If too much water is used, this phase becomes replaced by *Juncus balticus*. The only species in this phase that in any way makes up for the loss of *Deschampsia caespitosa* is *Scirpus microcarpus*. If the farmer allows the *Juncus* phase to enter, he should consider that his meadow has reverted. The next step is the bog, which would mean the destruc-

<sup>1</sup> Knight, H. G., Hepner, F. E., and Nelson, Aven. Op. cit.

tion of the meadow. However, nature works just the other way. Aquatic plants tend to fill up the bogs (see p. 758); then come the species of the *J. balticus* phase. Besides being so much less nutritious than the *D. caespitosa*, the hay of this phase will not average a ton to the acre; often only half a ton.

Now compare the man-made natural meadow with the nature-made natural meadow. If the highest type of the former, the Deschampsia stage, be taken as the standard, it far excels the natural condition. This is because the latter is usually that mixed type to which the Deschampsia phase tends, perhaps always ultimately reaches. *Deschampsia caespitosa* may be the most conspicuous plant in a natural meadow, but it rarely, if ever, is truly dominant over any considerable area. It is more likely to contain plants which are either useless or actually harmful and must be regarded as meadow weeds. The yield of the natural meadow is seldom as high to the acre as that of the Deschampsia phase of the artificial. On the other hand, the rush-sedge and *Juncus balticus* phases often have their counterparts in the natural meadow, so that by producing these the farmer gains nothing more than an increased acreage. So long as the Deschampsia phase can be maintained, not only a greater quantity but also a better quality of natural meadow hay has been obtained.

#### MEADOW HAY VERSUS ALFALFA AND GRAIN

In the spring of 1914 two tracts on the upland of this ranch, which so largely had been converted to meadow, were sown, one to alfalfa and the other to oats. The land was improperly prepared, and the distribution of water was uneven and poorly regulated. In spite of these obvious drawbacks, the two fields had produced by the end of the season the crops shown in Plate CV. It had been impossible to obtain definite data from the foreman of the ranch, but the yield of oats probably reached 30 bushels to the acre. Considering the way in which they were grown, this is a good yield. The illustration shows the excellent stand of alfalfa. A field on a neighboring ranch which was partly dry-farmed, there being water only during the fore part of the season, produced between 3 and 4 tons of alfalfa to the acre. If we recall the large number of native legumes the upland supported, we will not be surprised at the success of this legume, a plant notoriously fond of sweet soils rich in lime.

Plate CV, figure 1, shows the upland as it usually looks the first season under water, the strong development of *Antennaria parvifolia* being noteworthy. It is here that *Arabis hirsuta* flourished and became weedlike.

It must not be concluded that the artificial raising of natural hay does not pay. There are factors to be reckoned with which have not yet been considered. In the first place, it must be remembered that a natural meadow, once established, is fixed so long as the water supply holds out. On the other hand, alfalfa at this altitude needs reseeding every few



years, and oats often fail to mature. Besides, grain can not be grown indefinitely on the same land, even virgin land, without rotation. In the second place, the expenses of growing the cultivated crops are far greater than those of growing the uncultivated. The farmer who grows a natural meadow even by controlled irrigation as outlined above, ultimately will have less expense than the farmer who grows grain or alfalfa and continually has to repair ditches and regulate the water supply. Besides, he has not only been saved the initial cost of buying seed and of preparing a seed bed but he has also been able to utilize his entire upland, for all of it is potentially a meadowland.

These conclusions apply only to farming under the climatic conditions that exist at Rock Creek. At lower altitudes, where conditions are less rigorous and where cultivated crops well suited to the region have been long grown, this method of raising hay can not be too strongly condemned. It could be used with success only where hundreds of acres were available.

Even at Rock Creek on a smaller scale it would mean a criminal waste of land and water, as the ranch company is even now drawing so heavily on its reservoirs and on Rock Creek that during a dry season some of its meadows suffer. The owners have about reached the limit of increasing their hay yield by the growth of more natural meadows. Alfalfa gave much greater yields than the natural vegetation, and Briggs and Shantz have shown that alfalfa has a water requirement of only 831 units for every unit of dry weight produced. Further augmentation can come only by the raising of crops that require less water and give greater yields to the acre in return. The recent perfecting of grains and hays adapted to the high altitude and short season of the Laramie Plains will soon make this method of farming infeasible even under such conditions as those of Rock Creek.

#### MINOR PLANTS

Below are lists of those plants occurring in the Rock Creek region which have not been mentioned in the body of this paper, being mostly of interest to botanists only.

#### BENCH LAND

<i>Allium cernuum</i> Roth.	<i>Euphorbia montana</i> Engelm.
<i>Allium textile</i> Nels. and Macbr.	<i>Opuntia polyacantha</i> Haw.
<i>Eriogonum ovalifolium</i> Nutt.	<i>Cogswellia orientalis</i> Jones.
<i>Eriogonum flavum</i> Nutt.	<i>Gilia pungens</i> (Torr.) Benth.
<i>Paronychia sessilifolia</i> Nutt., var. <i>brevi-</i>	<i>Gilia spicata</i> Nutt.
<i>cuspis</i> A. Nels.	<i>Phlox glabrata</i> (E. Nels.) Brand.
<i>Lesquerella condensata</i> A. Nels.	<i>Oreocarya flavoculata</i> A. Nels.
<i>Lesquerella montana</i> (Gray) Wats.	<i>Oreocarya thyrsoiflora</i> Greene.
<i>Astragalus Drummondii</i> Dougl.	<i>Chrysopsis villosa</i> Nutt.
<i>Astragalus missouriensis</i> Nutt.	<i>Erigeron Eatonii</i> Gray.
<i>Astragalus nitidus</i> Dougl.	<i>Sideranthus grindelioides</i> (Nutt.) Britton.
<i>Astragalus Purshii</i> Dougl.	<i>Stenotus acaulis</i> Nutt.
<i>Astragalus Shortianus</i> Nutt.	

## DRAWS

<i>Calochortus Gunnisonii</i> Wats.	<i>Astragalus pectinatus</i> Dougl.
<i>Calochortus Watsonii</i> Jones.	<i>Astragalus succulentus</i> Rich.
<i>Eriogonum umbellatum</i> Torr., var. <i>intectum</i> A. Nels.	<i>Viola Nuttallii</i> Pursh.
<i>Eriogonum campanulatum</i> Nutt.	<i>Lithospermum angustifolium</i> Michx.
<i>Arenaria congesta</i> Nutt.	<i>Mertensia brevistyla</i> Wats.
	<i>Senecio perplexus</i> A. Nels.

## BENCH SLOPE

<i>Comandra pallida</i> A. DC.	<i>Pentstemon strictus</i> Benth.
<i>Fragaria ovalis</i> (Lehm.) Rydb., var. <i>glauca</i> (Wats.) A. Nels.	<i>Achillea millefolium</i> L.
<i>Astragalus bisulcatus</i> (Hook.) Gray.	<i>Helianthus Nuttallii</i> T. and G.
<i>Astragalus carolinianus</i> L.	<i>Lygodesmia grandiflora</i> T. and G.
<i>Thermopsis arenosa</i> A. Nels.	<i>Amelanchier oreophila</i> A. Nels.
<i>Epilobium paniculatum</i> Nutt.	<i>Prunus demissa</i> (Nutt.) Dietr., var. <i>melanocarpa</i> A. Nels.
<i>Gilia pharnaceoides</i> Benth.	<i>Shepherdia argentea</i> Nutt.
<i>Pentstemon alpinus</i> Torr.	<i>Cornus stolonifera</i> Michx.
<i>Castilleja linariaefolia</i> Benth.	

## STREAM VALLEY

<i>Salix Bebbiana</i> Sarg.	<i>Salix Fendleriana</i> Anders.
<i>Salix caudata</i> Muhl., var. <i>Watsonii</i> Bebb.	<i>Populus fortissima</i> Nels. and Macbr.
<i>Salix fluviatilis</i> Nutt.	<i>Betula fontinalis</i> Sarg.
<i>Salix fluviatilis</i> Nutt., var. <i>exigua</i> (Nutt.) Sarg.	<i>Alnus tenuifolia</i> Nutt.

## AQUATIC PLANTS OF THE REGION

<i>Potamogeton perfoliatus</i> L.	<i>Callitriche palustris</i> L.
<i>Lemna minor</i> L.	<i>Utricularia vulgaris</i> L.
<i>Batrachium pantothrix</i> S. F. Gray, var.	

## WOODLAND SPECIES TENDING TO INVADE ADJACENT MEADOWS

<i>Carex aurea</i> Nutt.	<i>Gentiana affinis</i> Griseb.
<i>Juncus nevadensis</i> Wats.	<i>Castilleja sulphurea</i> Rydb.
<i>Arabis columbiana</i> Macoun.	<i>Galium boreale</i> L.
<i>Cardamine Brewerii</i> Wats.	<i>Campanula rotundifolia</i> L.
<i>Oxytropis Lambertii</i> Pursh.	<i>Erigeron asper</i> Nutt.
<i>Geranium Richardsonii</i> F. and M.	<i>Senecio crocatus</i> Rydb.
<i>Gentiana acuta</i> , var. <i>strictiflora</i> Rydb.	

## MEADOW PLANTS APPEARING AT SOME LATER SEASON

<i>Equisetum hiemale</i> L.	<i>Parnassia parviflora</i> DC.
<i>Triglochin maritima</i> L.	<i>Argentina anserina</i> (L.) Rydb.
<i>Triglochin palustris</i> L.	<i>Thermopsis divaricata</i> A. Nels.
<i>Alopecurus fulvus</i> Smith.	<i>Vicia linearis</i> (Nutt.) Greene.
<i>Beckmannia erucaeformis</i> (L.) Host.	<i>Sidalcea neomexicana</i> Gray.
<i>Carex Douglasii</i> Boott.	<i>Cicuta occidentalis</i> Greene.
<i>Carex vaticola</i> Dewey.	<i>Zizia cordata</i> (Walt.) Koch.
<i>Juncus tenuis</i> Willd.	<i>Dodecatheon pauciflorum</i> (Durand) Greene.
<i>Allium Nuttallii</i> Wats.	<i>Primula farinosa</i> , var. <i>incana</i> (Jones) Fernald.
<i>Iris missouriensis</i> Nutt.	<i>Pedicularis crenulata</i> Benth.
<i>Sisyrinchium idahoense</i> Bickn.	<i>Carduus foliosus</i> Hook.
<i>Habenaria borealis</i> Cham.	<i>Crepis runcinata</i> (James) T. and G.
<i>Erysimum cheiranthoides</i> L.	

## SUMMARY

The artificial formation of natural meadows is a gradual change divisible into several distinct periods or phases, each characterized by one or more particular species.

The relative permanence of these stages may be largely controlled by regulation of the water supply. By the same means any stage, in some measure, may be produced at will.

*Agropyron* spp. and *Deschampsia caespitosa* furnish the most valuable hay.

The yield of the natural meadowlands is generally smaller and less nutritious than the possible yield of the artificial. The latter meadows, however, tend ultimately to be composed of the same type of vegetation that characterizes the natural meadows; therefore it is important to watch and control their development.

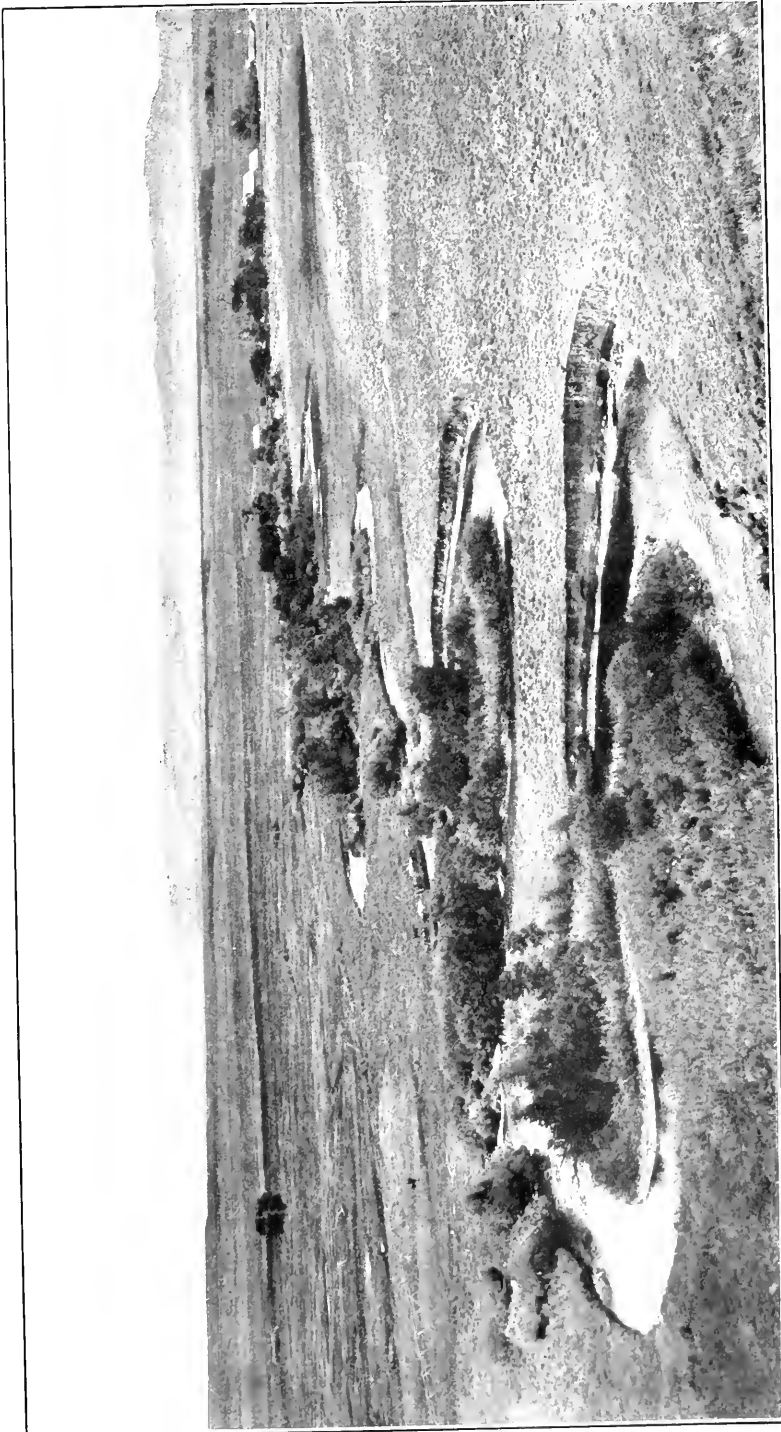
This manner of hay raising is practicable at high altitudes where both land and water are abundant and domestic crops are uncertain. Where conditions are favorable for cultivated crops, the method would be wasteful and should be regarded as unsound farming.

With the growing scarcity of farming land and the development of crops suited to areas usually considered nonarable, the practical artificial formation of natural meadows will become limited to regions of even higher altitude and shorter season than those considered in this paper.

PLATE XCVIII

Rock Creek Valley, near Rock River Station. Photographed by Dr. N. H. Darton, of the United States Geological Survey.

(760)



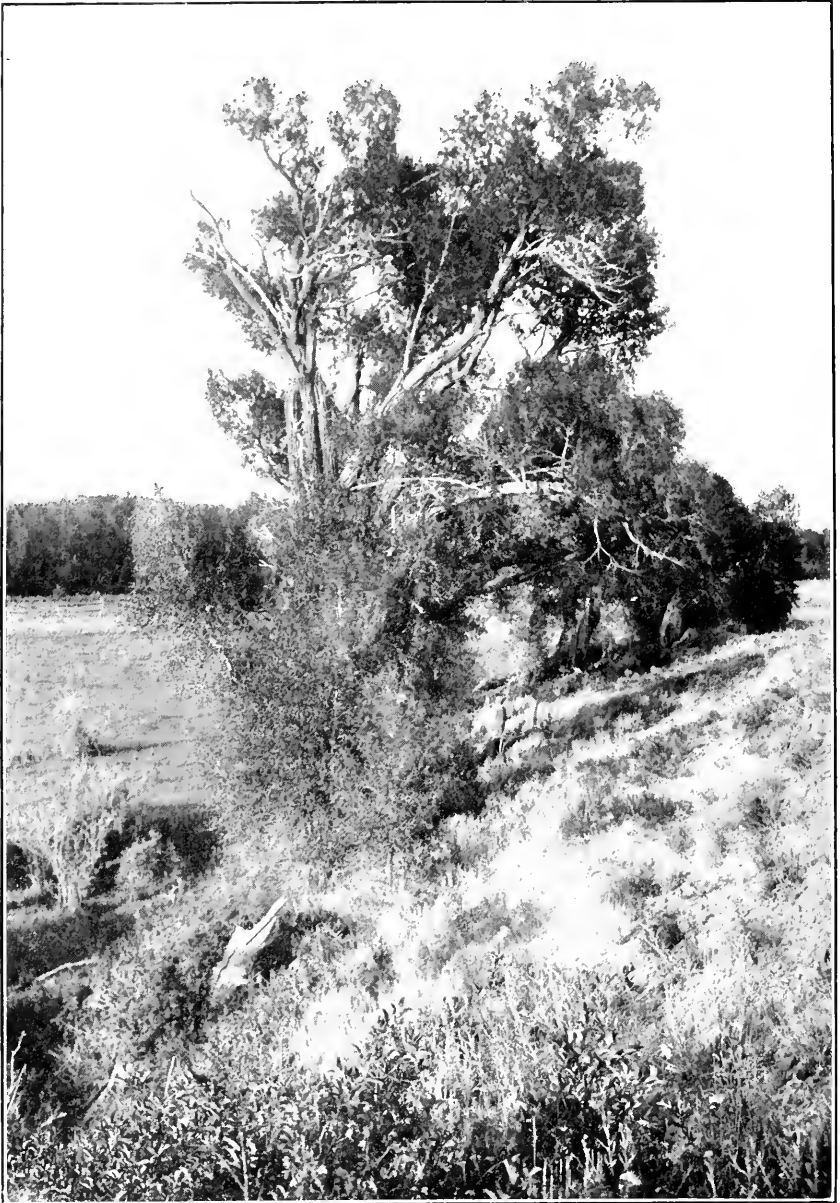


PLATE XCIX

A nearer view of the bench slope; the same tree shown in Plate C, figure 1.

PLATE C

Fig. 1.—Where upland and lowland meet. Notice the bench slope; described on page 745. For list of shrubs see "Minor plants, pages 757-758. The cottonwood tree is *Populus fortissima* Nels. and Macbr.

Fig. 2.—Characteristic draw; the stream valley beyond. Lupin, wheat-grass, white sage, and gaillardia in the foreground.





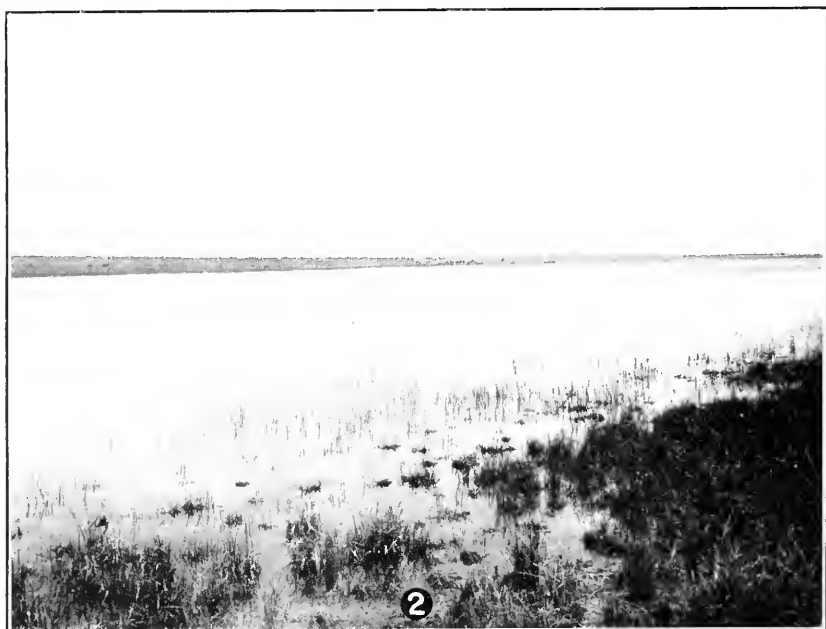


PLATE CI

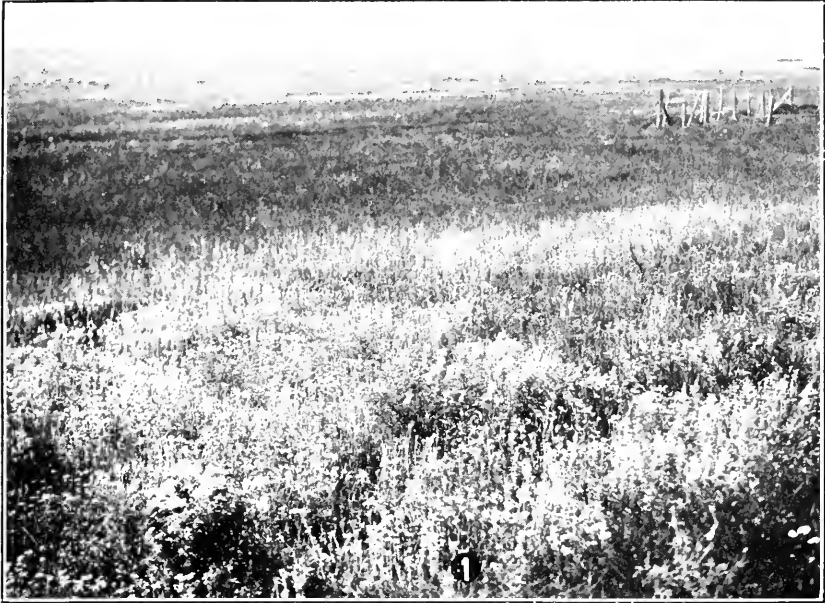
Fig. 1.—The bench. The course of Rock Creek is indicated by the distant trees.

Fig. 2.—Part of a reservoir on the Rock Creek ranch.

PLATE CII

Fig. 1.—Lupin recessive and cat's-foot becoming dominant. Gay's sedge subphase in background.

Fig. 2.—Wheat-grass phase. Notice the occasional squirrel-tail grass.



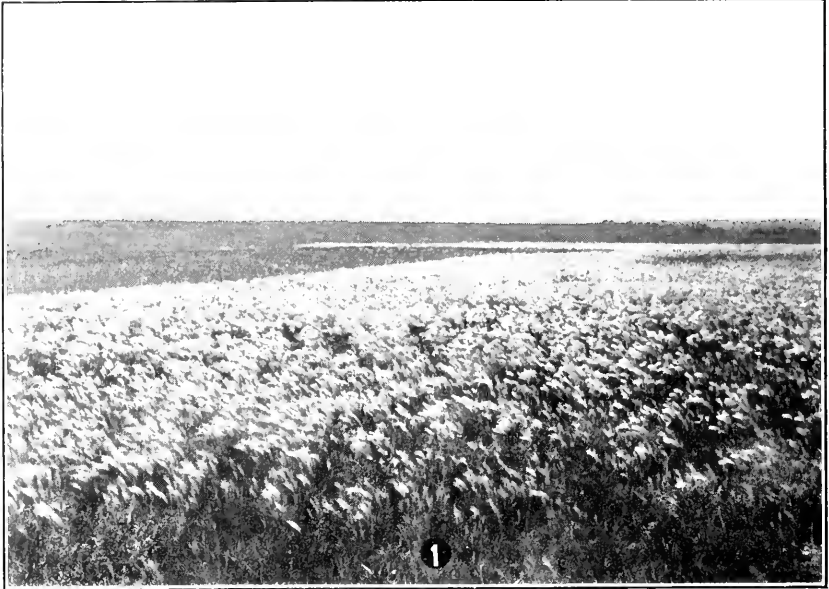


PLATE CIII

Fig. 1.—Squirrel-tail phase. A few grindelias in the foreground.

Fig. 2.—Rush-sedge phase (the darker areas) replacing wheat-grass phase.

PLATE CIV

Fig. 1.—Hair-grass phase.

Fig. 2.—Natural meadow. Note the cosmopolitan character of vegetation.





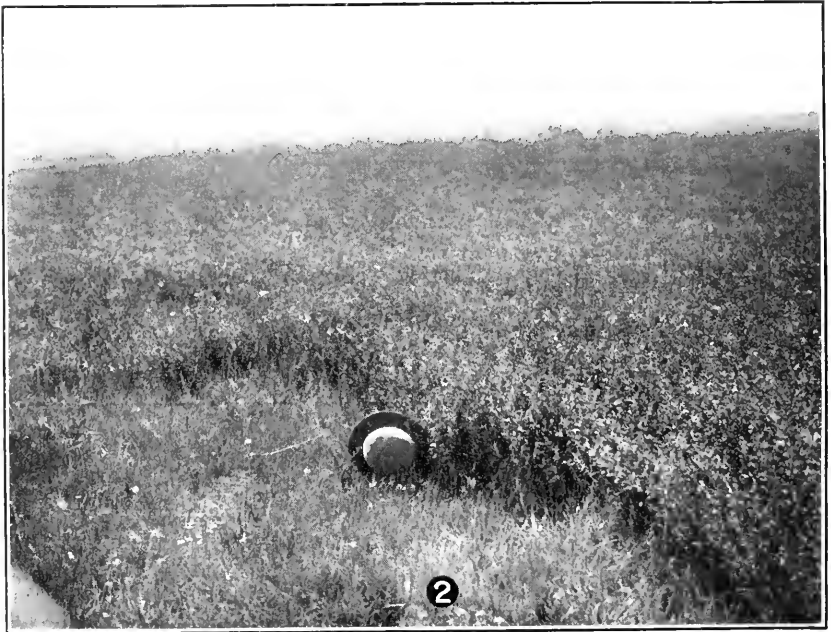
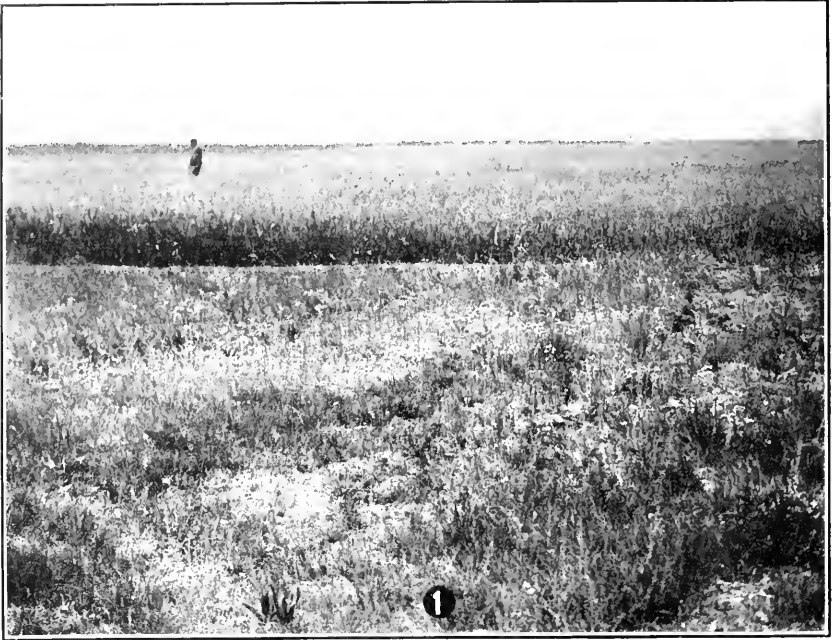


PLATE CV

Fig. 1.—Field of oats on bench. Cat's-foot and other upland plants in foreground.

Fig. 2.—Alfalfa field one year after sowing. Cat's-foot and bench grasses in foreground.

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## AGRICULTURAL VALUE OF IMPERMEABLE SEEDS

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### INTRODUCTION

During the years 1909 to 1916, inclusive, many germination tests of lots of clover and alfalfa seed were made for the purpose of determining the agricultural value of the impermeable seeds. A smaller number of tests of winter vetch, okra, and other seeds were made for the same purpose.

Impermeable seeds, in the sense in which the term is used in this paper, are seeds whose coats are impermeable to water at temperatures favorable for germination. In the majority of plants which produce impermeable seeds this feature of the seed coat results from the peculiar character of its outer layer of cells, which may, in addition, be covered by a continuous cuticle.

Such seeds have been described by numerous investigators, including the present author, under the term "hard seeds." Guppy (9)<sup>1</sup> has, however, introduced a more appropriate term, "impermeable seeds," which will be used in the present paper. The term is relative, as impermeable seeds are capable of becoming permeable. While in the impermeable condition they remain very hard and dry, even when surrounded by water. When they become permeable, they absorb water readily, becoming soft and swollen. Naturally no seed can germinate while in the impermeable condition. In speaking of the germination of impermeable seeds, therefore, one means simply the germination of seeds which were impermeable at some previous time.

Many species of plants produce both impermeable seeds and seeds whose coats are readily permeable to water at one or more points. According to Guppy (9), these two types of seed can easily be distinguished by structural differences in certain plants (*Entada polystachya* and *Axyris amaranthoides*), but this is not true of any of the plants considered in this paper.

Verschaffelt (18) has investigated the relative permeability to both water and other liquids of different areas of the seed coats of a large

<sup>1</sup> Reference is made by number to "Literature cited," p. 796.

number of plants which produce impermeable seeds. The present author has found that many of the seeds of the species of plants considered in this paper are more readily permeable to water in the region of the chalaza than elsewhere.

Nobbe and Haenlein (16), Gola (8), Ewart (7), Rees (17), and Guppy (9) have given us a good idea of the distribution among the natural plant families of species which produce impermeable seeds. All agree upon the Leguminosae as far surpassing all other families in this respect. Many other families also contain species which produce impermeable seeds.

#### OCURRENCE OF IMPERMEABLE SEEDS IN CULTIVATED SPECIES

The peanut (*Arachis hypogaea*) excepted, probably all commercially important legumes cultivated in the United States produce a greater or less percentage of impermeable seeds. The percentage is small or fails entirely with spring vetch (*Vicia sativa* L.), some varieties of soybeans (*Soja max* (L.) Piper), kidney beans (*Phaseolus vulgaris* L.), Lima beans (*Phaseolus lunatus* L.), garden peas (*Pisum sativum* L.), and the newly introduced black bitter vetch (*Vicia ervilia* (L.) Willd.).

Table I shows the percentage of impermeable seeds in the commercial samples of a number of small-seeded legumes which have been tested by the Seed Laboratory during the six years 1904 to 1909.

TABLE I.—Percentages of impermeable seeds in small-seeded legumes tested during the years 1904 to 1909

Kind of seed.	Number of lots tested.	Percentage of impermeable seeds.		
		Maximum.	Minimum.	Average.
Red clover ( <i>Trifolium pratense</i> L.).....	1, 642	46	0	9. 61
Alsike clover ( <i>Trifolium hybridum</i> L.).....	304	40	0	10. 16
White clover ( <i>Trifolium repense</i> L.).....	125	38	0	17. 30
White sweet clover ( <i>Melilotus alba</i> Desv.)	37	87	1. 5	42. 39
Alfalfa ( <i>Medicago sativa</i> L.).....	1, 737	72	0	13. 81
Winter vetch ( <i>Vicia villosa</i> Roth.).....	30	68	0.	20. 97
Spring vetch ( <i>Vicia sativa</i> L.).....	28	8	0	0. 96
Cowpea ( <i>Vigna sinensis</i> (Tornier)Savi) <sup>1</sup> ..	37	60	0	3. 55
Toothed bur clover ( <i>Medicago hispida denticulata</i> (Willd) Urban).....	6	85	9	48. 08
Spotted bur clover ( <i>Medicago arbica</i> (L.) Huds.) <sup>2</sup> .....	9	92	35	71. 67
Yellow-flowered sickle lucern ( <i>Medicago sativa falcata</i> (L.) Döll) <sup>1</sup> .....	5	86	35. 5	49. 72
Yellow trefoil ( <i>Medicago lupulina</i> L.)....	10	46	0	10. 45

<sup>1</sup> Only two years' tests.

<sup>2</sup> Only one year's tests.

Recent investigations by the author (10) show that the first four species of plants in Table I produce very much higher percentages of impermeable seeds than are indicated and that many of the seed coats become permeable to water during the operation of hulling the seeds.

Important nonleguminous plants which produce impermeable seeds are okra (*Hibiscus esculentus* L.) and hollyhock (*Althea rosea* (L.) Cav.), both



belonging to the Malvaceae, atriplex (*Atriplex* spp.), of the Chenopodiaceae, alfilaria (*Erodium cicutarium* (L.) L'Hér.), of the Geraniaceae, asparagus (*Asparagus officinalis* L.), of the Convallariaceae, morning-glory (*Ipomea purpurea* (L.) Lam.), of the Convolvulaceae, and canna (*Canna indica* L.), of the Cannaceae. The cherry-tomato (*Physalis pubescens* L.), of the Solanaceae, occasionally has some impermeable seeds.

#### LONGEVITY OF IMPERMEABLE SEEDS

Although stating that some seeds with readily permeable coats may retain their vitality for many years in dry air, Ewart (7) was inclined to attribute extreme longevity of seeds in the soil exclusively to the impermeability of the seed coats. In contrast to Ewart's conclusion the work of Duvel,<sup>1</sup> Beal (1-5), and others indicates that great longevity of seeds even in moist soil may sometimes be the result of factors entirely independent of an impermeable seed coat. There is no doubt, however, that the possession of such a seed coat contributes to the length of life of the seed by decreasing or entirely preventing respiration and imbibition and, in general, by reducing the rapidity with which all physical and chemical changes take place within the seed. The seeds which Ewart (7) and Rees (17) have shown to retain their viability for 15 to 50 years or longer are almost exclusively seeds with impermeable seed coats. Beal, however, found the seeds of some typically permeable seeds (for example, *Brassica nigra*) viable after 30 years in the soil.

Becquerel (6) has reported the germination of impermeable seeds of three leguminous plants over 80 years old, and Ewart (7) mentions several plants as germinating from 5 to 80 per cent when over 50 years old.

According to Ewart, the curves of viability based on the germinating capacity of seeds of known age suggest 150 to 250 years as the probable extreme longevity of any known seed.

#### EXPERIMENTAL WORK DURING 1909-1916

The major part of the work reported in this paper was done with the seeds of red clover, alsike clover, white clover, white sweet clover, alfalfa, hairy vetch (*Vicia villosa* Roth.), and okra. A small amount of work was done also with seeds of crimson clover (*Trifolium incarnatum* L.), black locust (*Robinia pseudacacia* L.), kidney bean (*Phaseolus vulgaris* L.), garden and field peas (*Pisum sativum* L.), cowpeas, and *Chamaecrista nictitans* L. Muench.

Nearly all chamber tests and greenhouse tests were made with two samples of 100 seeds each from each lot of seed tested. In some cases the number available was small and less than 200 seeds were used. A number of tests of okra were made with two lots of 50 seeds each. In the

<sup>1</sup> Unpublished data.

field from 250 to 500 seeds were used in testing each lot, 500 being tested in nearly every case.

In all of the germination tests which were conducted in the germinating chambers folded blue blotting paper free from soluble dye was used as germinating beds for the seeds of the clovers, alfalfa, black locust, and *Chamaecrista nictans*, and folded canton flannel for the seeds of the other kinds of plants. In the green house tests both sand and steam-sterilized potting soil were used.

#### VIABILITY OF IMPERMEABLE SEEDS

In May and June, 1914, 128 lots of seed from 1 to 5 years old were tested for germination, and the viability of the seeds remaining impermeable after six days was determined and compared with the viability of the seeds which softened in the first six days of the test.<sup>1</sup> Table II summarizes the results.

TABLE II.—*Viability of impermeable seeds from 1 to 5 years old*

Kind and age of seed.	Number of lots.	Average percentage of—			
		Germination.	Impermeable seeds.	Viability of impermeable seeds.	Viability of seeds which softened in six days.
Red clover:					
5 years.....	21	34	59	99	83
4 years.....	5	43	43	96	75
3 years.....	9	34	58	99	81
2 years.....	11	39	60	99	98
1 year.....	8	26	74	100	99
Alsike clover:					
3 years.....	4	15	81	100	79
2 years.....	6	7	90	100	70
1 year.....	5	6	90	100	60
White clover:					
3 years.....	1	8	82	95	44
2 years.....	6	8	90	100	80
1 year.....	4	12	87	100	92
Sweet clover:					
4 years.....	2	1.5	98	100	75
2 years.....	12	4	95	98	80
1 year.....	3	10	89	100	91
Alfalfa:					
3 years.....	6	71	25	100	95
2 years.....	5	60	28	100	96
1 year.....	7	67	32	100	99
Hairy vetch:					
5 years.....	1	57	8	100	61
1 year.....	3	59	17	100	71
Crimson clover: 2 years.....	2	65	26	100	88
Okra: 3 years.....	5	9	87	92	69
<i>Chamaecrista nictans</i> :					
4 years.....	1	36	43	85	63
<i>Robinia pseudacacia</i> :					
At least 5 years.....	1	25	54	95	54

<sup>1</sup> To determine the viability of the impermeable seeds, the seed coats of 20 seeds from each lot (or all seeds remaining impermeable after six days if not more than 20) were cut with a knife, and these seeds with cut seed coats were then subjected to germination conditions for seven days.

Over 90 per cent of the impermeable seeds were viable in every case, except the lot of seed of *Chamaecrista nictans*. In most cases 100 per cent were viable. The average percentage of viability of the impermeable seeds was invariably greater than of the seeds which softened within six days. The difference ranged from 1 per cent to over 50 per cent—the latter with 3-year-old white-clover seed—and in general increased with the age of the seed.

RATE OF SOFTENING OF IMPERMEABLE SEEDS WHEN KEPT IN WET BLOTTERS

Table III shows the average rates of softening of seeds which had remained impermeable after 10 days in wet blotters. These seeds were kept in wet blotters for three years.

TABLE III.—Rate of softening of impermeable seeds when kept in wet blotters

Kind of seed.	Description. <sup>1</sup>	No. of lots.	Average percentage of seeds impermeable after 10 days.	Average percentage of impermeable seeds as shown in preceding column which softened in time indicated.			
				1 month.	1 year.	2 years.	3 years.
Red clover.....	Hand-gathered.....	20	86	8	30	44	55
Do.....	Commercial.....	6	11	9	27	45	55
Alsike clover.....	Hand-gathered.....	2	91	9	52	63	66
Do.....	Commercial.....	5	14	7	21	28	28
White clover.....	do.....	5	30	3	13	23	30
Sweet clover.....	Hand-gathered.....	2	97	5	27	35	44
Do.....	Commercial.....	4	24	8	21	29	37
Alfalfa.....	Hand-gathered.....	10	70	46	97	99	100
Do.....	Commercial.....	8	20	10	70	90	95
Hairy vetch.....	Hand-gathered.....	2	<sup>a</sup> 65	13	85	97	98
Crimson clover.....	do.....	2	45	42	93	100	.....
Do.....	Commercial.....	5	12	50	100	.....	.....
Okra.....	do.....	5	<sup>a</sup> 56	34	91	96	98

<sup>1</sup> All hand-gathered lots of seed were gathered and hulled by hand a few days before the beginning of the tests. Commercial lots were of uncertain age, probably in most cases a little less than 1 year old.

<sup>a</sup> After 15 days.

1. Less than 10 per cent of the seeds of red clover, alsike clover, white clover, and sweet clover which remained impermeable after 10 days softened in one month; and from about one-third to a little over one-half of them softened in three years.

2. Nearly all of the impermeable seeds of alfalfa, hairy vetch, okra, and crimson clover which remained after 10 days softened in one year in wet blotters, but a very few of all except crimson clover remained impermeable after three years. There is a marked contrast in this respect between these species of plants and those named in the preceding paragraph.

3. The impermeable seeds in hand-gathered lots of alsike-clover seed and alfalfa seed softened much more rapidly than the impermeable seeds

in commercial lots of seed of the same plants. There was but little difference in this respect between the impermeable seeds of hand-gathered lots and commercial lots of the other species of plants.

Almost all of the seeds of each kind of plant which softened at any time during the three years germinated and produced vigorous seedlings.

Besides seeds of the species given in Table II a few impermeable seeds of kidney bean, garden pea, and cowpea were included in the tests. All of the beans and peas softened and germinated within three months, and all of the cowpeas within eight months.

#### INFLUENCE OF MATURITY ON THE RATE OF SOFTENING OF IMPERMEABLE RED-CLOVER AND ALSIKE-CLOVER SEEDS IN WET BLOTTERS

The lots of hand-gathered seed included under Table III were thoroughly mature and dry in the heads before being removed from the plants. Figure 1 shows graphically the comparative rates of softening of such well-matured impermeable red-clover seed and of impermeable red-clover seed of two other degrees of maturity.

Seven of the eight lots of slightly immature seed were gathered at the same time and from the same cultivated rows of plants as were seven of the eight lots of mature seed, the former being taken from heads which were slightly green and succulent, the latter from black, dry heads. The three lots of light, immature seed used in the comparison were separated from three of the lots of slightly immature seed by a gravity blowing machine. Only seeds of good appearance, though frequently of small size in the immature lots, were used in the tests.

The average percentages of the mature seeds, the slightly immature seeds, and the more immature seeds which remained impermeable after 10 days in wet blotters and from which the rates of softening were calculated were respectively 84, 72, and 27.

Of the impermeable seeds from lots of light, immature seed, 78 per cent softened in one month and 100 per cent in 13 months. In contrast to this, only 5 per cent of the impermeable seeds from lots of well-matured seed softened in one month and 44 per cent of them remained impermeable after three years.

The differences in the rate of softening of impermeable seeds from lots of mature and immature seeds of alsike clover were similar to those shown for red-clover seed. No other species of plants were investigated for rate of softening.

Hiltner (11) has shown that the percentage of impermeable seeds and the rate at which they soften when placed under conditions favorable to imbibition may in some cases be greatly altered by previous drying. Although only seeds which seemed to be thoroughly dry were used in these experiments, it is possible that artificial desiccation of the less mature lots would have increased the percentages of impermeable seeds and de-

creased the rate at which they softened. As will be shown later, however (p. 775), moderate heating of thoroughly air-dried seeds of these plants has little or no effect under ordinary conditions.

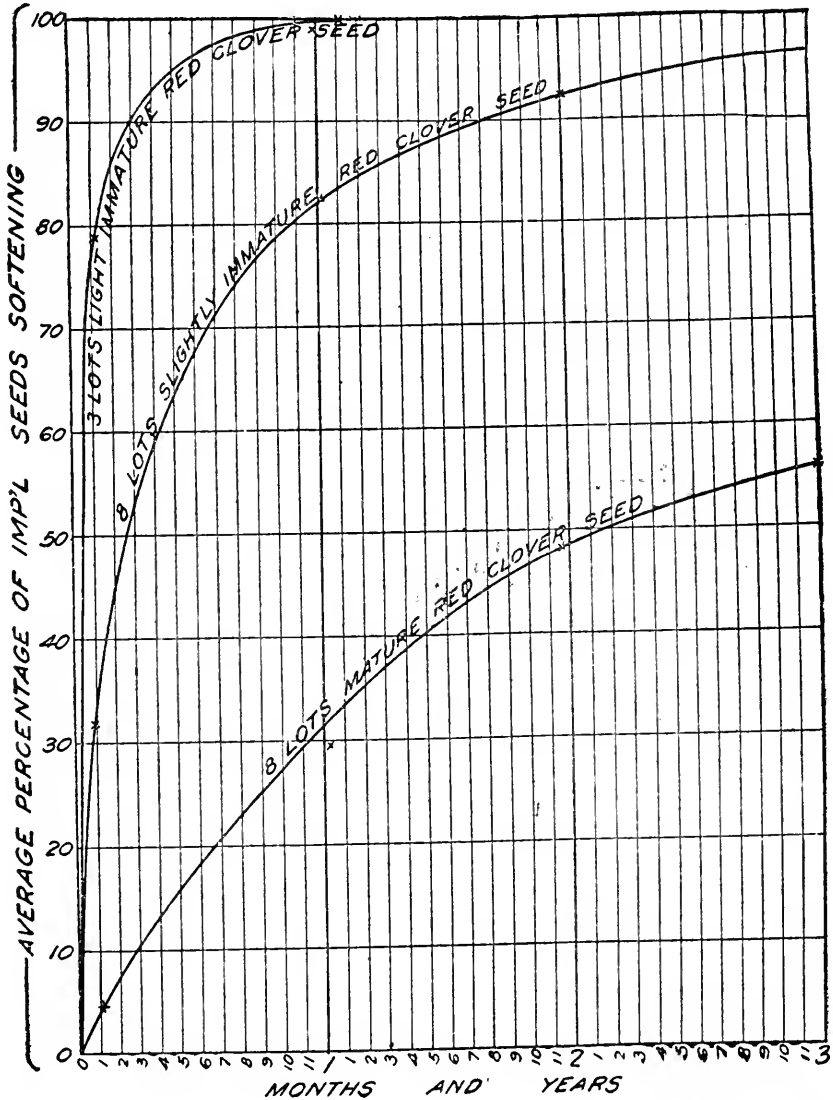


FIG. 1.—Curves showing the rate of softening of impermeable red-clover seeds of different degrees of maturity.

ESTIMATION OF THE GERMINABILITY OF IMPERMEABLE SEEDS

It is evident from the preceding discussion that it is impossible to estimate in advance what proportion of the impermeable seeds of a given lot will germinate under ordinary germination conditions in any given length of time. At one time Nobbe (14) proposed that one-third

of the seeds of red clover remaining impermeable after 10 days be reckoned as capable of germinating in one year. He later tested in distilled water 66 lots of red-clover seed from various sources, using 1,000 seeds of each lot. From 2.4 to 90 per cent of the seeds of the different lots which remained impermeable after 10 days softened in

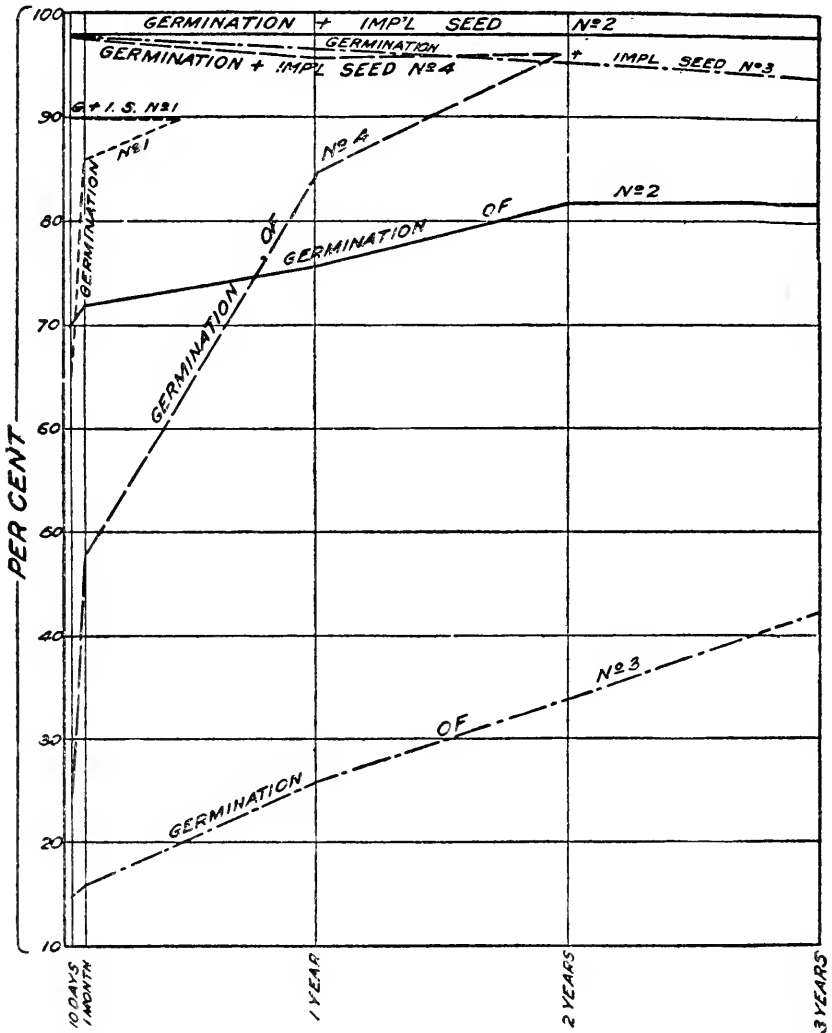


FIG. 2.—Curves showing the rate of softening and of germination of impermeable red-clover seeds of different lots.

distilled water in one year. On the basis of these results Nobbe (15) abandoned his previous position as untenable.

Different lots of seeds of any one of the species of plants included in Table III illustrate differences in the rate of softening of the impermeable seeds which are comparable to those reported by Nobbe. Figure 2

shows graphically the differences in this respect between four lots of red-clover seed. Lot 2 was commercial seed, while the other lots were seed which had been gathered and hulled by hand. While lot 3 was thoroughly dry when gathered, lots 1 and 4 were gathered when the heads were slightly green.

The lower of each pair of lines indicates the progress of germination of one lot of seed, the upper line the sum of percentages of germination and of impermeable seeds, and the space between the two lines of the pair the percentage of seeds which were impermeable at any given time.

1. All of the seeds of lots 1 and 4 softened and practically all germinated in, respectively, six months and two years. Yet only about 40 per cent of the impermeable seeds of lots 2 and 3 softened and germinated in three years.

2. Since the percentages of seeds of lots 1 and 4 which remained impermeable during the first 10 days were about the same as of lots 2 and 3, it is evident that the original percentage of impermeable seeds bears no relation to the rate at which these will soften or germinate.

3. It should be emphasized that the differences in maturity of the different lots of seed were not noticeable in the appearance of the seeds and offered, therefore, no basis for estimating the percentages of impermeable seeds which the different lots contained or the rates at which these impermeable seeds would soften.

RATE AT WHICH IMPERMEABLE SEEDS BECOME PERMEABLE WHEN STORED IN MANILA ENVELOPES

Hand-gathered, hand-hulled seeds were tested for germination and impermeable seeds after being stored in manila envelopes for different lengths of time. The first test of each lot was begun a few days after the seeds were harvested, and the last test in some cases more than 4½ years later.

Table IV shows the calculated average percentages of the originally impermeable seeds in some of these lots of seed which became permeable in one month, one year, two years, three years, and four years. Loss of permeability during storage is indicated by the minus (-) sign.

1. The impermeable seeds of red clover, alsike clover, white clover, and sweet clover became permeable very slowly in dry storage. The red-clover seed changed more rapidly than the other kinds of clover seed, but less than one-half of the impermeable seeds of this species became permeable in four years.

2. The percentages of impermeable seeds in lots of alsike-clover, white-clover, and sweet-clover seed gathered in 1912 increased slightly during the first year and then remained about constant during the second year. This initial increase is probably due to the seed's being tested the first time before it was thoroughly dry. A similar increase occurred in a few lots of red-clover and alfalfa seed.

TABLE IV.—Rate at which impermeable seeds became permeable when stored in manila envelopes

Kind of seed.	Year in which grown.	Number of lots.	Average percentage of impermeable seeds when gathered.	Calculated average percentage of the impermeable seeds as shown in preceding column which became permeable in time indicated.				
				1 month.	1 year.	2 years.	3 years.	4 years.
Red clover.....	1909	12	80	0	.....	.....	33	35
	1910	5	81	.....	14	41	.....	48
	1911	9	86	.....	22	.....	34	.....
Alsike clover.....	1911	4	85	.....	9	.....	6	.....
	1912	6	86	-2	.....	-3	.....	.....
White clover.....	1912	6	88	.....	-5	-2	.....	.....
Sweet clover.....	1912	12	85	.....	-12	-12	.....	.....
Alfalfa.....	1911	6	80	.....	24	70	.....	.....
	1912	4	60	2	.....	55	.....	.....
Hairy vetch.....	1909	1	73	.....	.....	.....	90	95
	1913	a 1	60	.....	82	.....	.....	.....

<sup>a</sup> This lot of hairy-vetch seed was grown by the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, at Chico, Cal.

3. Impermeable alfalfa and hairy-vetch seed <sup>1</sup> became permeable more rapidly than impermeable clover seed, 82 per cent of one lot of hairy-vetch seed becoming permeable in one year.

Besides the kinds of seed given in Table IV, five lots of okra seed gathered in 1911 were tested when fresh and six months, two years, and three years later. When fresh, all seeds softened and an average of 98 per cent germinated. Six months later only 23 per cent germinated and 71 per cent were impermeable. During the following two and one-half years very little change in permeability occurred.

It should be added that there was a slight decrease in the viability of the red-clover seed during the third and fourth years, and a large decrease in the viability of the vetch seed during the fourth year.

#### VARIATION IN THE RATE AT WHICH IMPERMEABLE SEEDS OF A SINGLE SPECIES BECOME PERMEABLE

The impermeable seeds in some of the lots included in Table IV became permeable in dry storage much more rapidly than those of other lots of the same kinds of plants. In fact, the percentage of the impermeable seeds in different lots of red-clover seed which became permeable in four years varied from about 15 to about 80. This variation is further emphasized by the results of tests conducted in the fall and winter of 1914-15 and in September and December, 1915, using

<sup>1</sup> The vetch seeds in the lots here considered were thoroughly dry and black when first tested. Vetch seed, while it remains green in color and has a high water content, contains but a small percentage of impermeable seed or none. This percentage increases in storage for a time before any decrease takes place.



exclusively seeds grown in 1914.<sup>1</sup> Table V shows the average percentages of the viable seeds which were impermeable in the two tests and the calculated average and maximum percentages of the seeds, impermeable when freshly gathered, which became permeable in the interval of about one year between the two tests.

TABLE V.—Change in permeability of clover and alfalfa seeds during the first year after harvesting

Kind of seed.	Manner of hulling.	Number of samples.	Average percentage of viable seeds which were impermeable.		Calculated percentages of the seeds, impermeable when fresh, which became permeable in about 1 year.	
			Fresh.	1 year old.	Average.	Maximum.
Red clover.....	Hand. ....	220	92	87	6	52
Do.....	Machine.	207	17	14	19	140
Alsike clover.....	Hand. ....	12	91	91	0	.....
Do.....	Machine.	37	18	16	10	126
White clover.....	Hand. ....	8	98	90	8	55
Do.....	Machine.	5	34	28	18	140
Sweet clover.....	Hand. ....	6	98	98	0	.....
Alfalfa.....	Machine.	2	32	7	60	87

<sup>1</sup> These calculations are based only on lots of which 30 per cent or more were impermeable when tested the first time.

The hand-hulled lots contained very large percentages and the machine-hulled lots comparatively small percentages of impermeable seeds. With few exceptions in the case of single lots of seed, the impermeable seeds in the hand-hulled lots became permeable more slowly than those in the machine-hulled lots.

The average results for all lots showed that not more than 8 per cent of the impermeable seeds in hand-hulled lots of the different kinds of clover seed had become permeable during the interval between the two tests; yet over 50 per cent of the impermeable seeds in one lot each of hand-hulled red-clover seed and hand-hulled white-clover seed became permeable.

The case of the hand-hulled white-clover seed is especially interesting. Eight lots averaged 98 per cent of impermeable seeds when fresh. Only 1 per cent of the impermeable seeds in seven of these lots became permeable in a little over a year. Of the impermeable seeds in the other lot 36 per cent became permeable in two months, 45 per cent in three months, and 55 per cent in 14 months. Nothing in the appearance of the different lots of seed either of white clover or of the other kinds indicated that any differences would be found in the rate at which the impermeable seeds became permeable.

<sup>1</sup> The results of the special investigation on impermeable clover seed conducted in the fall and winter of 1914-15 have been published elsewhere (10).

It should be added that no change could be detected in the viability of the seeds during the interval between the two tests except in case of the machine-hulled lots of red-clover seed and of alfalfa seed. With these there was a very slight decrease in viability.

#### INFLUENCE OF MATURITY ON THE RATE AT WHICH IMPERMEABLE RED-CLOVER SEEDS BECOME PERMEABLE IN MANILA ENVELOPES

Four lots of red-clover seed were gathered in July, 1910. This was the earliest seed of a good grade that could be obtained from the plants in question. Four other lots of seed were gathered from the same cultivated rows of plants in October, 1910.

The average percentages of impermeable seeds in the lots of seed gathered in July and in October were, respectively, 71 and 82. When again tested for germination two years later, the average percentages of impermeable seeds were 35 and 62. In other words, one-half of the impermeable seeds in the less mature lots which were gathered in July and one-fourth of those in the more mature lots which were gathered in October became permeable in two years. There was practically no change in viability during the two years.

#### COMPARATIVE RATES AT WHICH IMPERMEABLE SEEDS BECOME PERMEABLE IN WET BLOTTERS AND IN DRY STORAGE

A comparison of Tables III and IV shows that impermeable seeds become permeable more rapidly when kept under germination conditions than when stored dry. The difference in rates varies widely among different lots of the same species.

Between 20 and 40 per cent more of the majority of the lots of hand-hulled red-clover seed remained impermeable after four years in dry storage than after four years in wet blotters. With a few lots of red-clover seed, however, the differences were less than 5 per cent, and with a few other lots the differences were between 50 and 60 per cent.

Figure 3 represents graphically the changes in the percentage of impermeable seeds of typical lots of red clover, alsike clover, sweet clover, and alfalfa seed when kept in wet blotters and when stored dry in manila envelopes for various periods.

1. With each species the percentage of impermeable seeds decreased more rapidly in wet blotters than in dry storage.
2. The percentage of impermeable seeds decreased more rapidly during the first year than during succeeding years both in wet blotters and in dry storage.

#### PRODUCTION OF SEEDLINGS IN SOIL BY IMPERMEABLE LEGUMINOUS SEEDS

Comparative tests were made in germinating chambers, in a greenhouse, and in soil outdoors, using lots of seed with varying percentages of impermeable seeds. The results of these tests indicate that, with rare

exceptions, very few of the impermeable seeds of the different kinds of clover, except crimson clover, will produce seedlings in the soil even in three months at temperatures such as prevail in late spring or in summer.

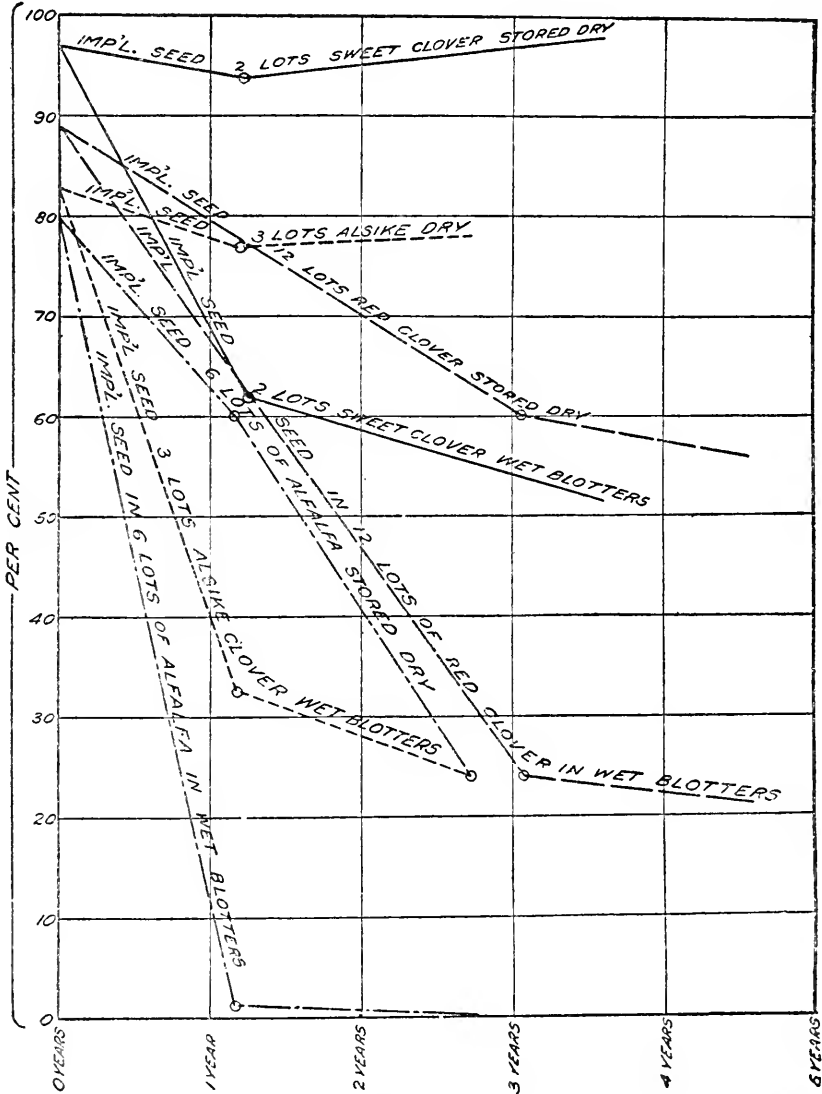


FIG. 3.—Curves showing the changes in the permeability of seeds in wet blotters and in dry storage for various periods.

The case of alfalfa, crimson clover, and the larger-seeded species is different. It was calculated that varying percentages of the impermeable seeds of alfalfa, hairy vetch, Canada field pea, cowpea, and okra produced seedlings in a few days or weeks both in a moderately warm

greenhouse and in greenhouse flats in which tests were conducted outdoors in warm weather.

In one experiment it was calculated that 16 per cent of the impermeable red-clover seeds, 5 per cent of the impermeable white-clover and sweet-clover seeds, and 38 per cent of the impermeable alfalfa seeds produced seedlings in greenhouse flats in three months. In the same time 5, 2, 3, and 8 per cent, respectively, germinated in a germinating chamber at room temperature, the rest remaining impermeable.

On June 7, 1909, seeds remaining impermeable during germination tests in germinating chambers were sowed in soil in large greenhouse flats, which were then set outdoors in the warm space between two greenhouses. The soil was kept watered, and observations were continued until November 30, 1909. During the latter half of November the temperature fell below freezing several times, but the last few days of the test were warm. The results of the tests are summarized in Table VI.

TABLE VI.—*Production of seedlings by leguminous seeds which remained impermeable during germination tests*

Kind of seed and test No.	Number of seeds used.	Percentage of seeds which produced seedlings in—			
		1 month.	2 months.	3 months.	6 months.
<b>Red clover:</b>					
58593.....	110	5	7	8	8
83583.....	109	12	16	17	28
83843.....	76	3	5	5	5
85272.....	87	5	7	7	9
85341.....	43	5	5	5	5
85371.....	30	17	23	23	37
85454.....	25	8	8	12	16
Average.....		6	10	11	14
<b>Sweet clover:</b>					
78539.....	195	3	4	4	<sup>a</sup> 14
<b>Alfalfa:</b>					
62874.....	12	75	92	92	92
78479.....	75	53	57	57	59
85276.....	30	80	90	90	90
Average.....		69	80	80	80
<b>Hairy vetch:</b>					
78326.....	24	38	38	38	38

<sup>a</sup> During the first 5 months of the experiment 4 per cent were produced; the remaining 10 per cent during the last few days of the experiment, following the freezing of the soil.

1. Only 14 per cent of the impermeable sweet-clover seeds and an average of 14 per cent of the impermeable red-clover seeds produced seedlings in six months.

2. An average of 69 per cent of the impermeable alfalfa seeds produced seedlings in one month, and an average of 80 per cent in two months, after which only one new seedling appeared in the next four months.

3. Thirty-eight per cent of the one lot of impermeable hairy-vetch seeds produced seedlings in one month, after which no new seedlings appeared.

4. It is worthy of notice that after four months, during which no new sweet-clover seedlings appeared, 10 per cent of the seeds used produced seedlings in a few days following the cold weather in November. This is particularly interesting in the light of subsequent results.

#### EFFECT OF DIFFERENT CONDITIONS UPON THE GERMINATION OF IMPERMEABLE SEEDS IN SOIL

It has been shown that some seeds which would be reported as impermeable according to the chamber tests will produce seedlings in a comparatively short time in the soil. Experiments were conducted to determine the effects of separate factors.

Comparative tests showed that neither moistening the blotters which were used for germinating bed with strong aqueous soil extracts nor the alternate wetting and drying of the seeds at frequent intervals affects the rate of softening of impermeable clover seeds.

Neither the depth of planting nor the firmness of the soil nor the conservation of surface moisture by shading affected the percentage of seedling production by impermeable clover and alfalfa seeds in greenhouse tests, except when the seeds were planted over  $\frac{3}{4}$  inch deep. Fewer seedlings reached the surface of the soil from seeds planted 1 inch deep than from seeds planted  $\frac{1}{4}$  to  $\frac{3}{4}$  inch deep.

On the other hand, certain factors influenced the softening of impermeable seeds and would probably affect the production of seedlings in the soil. These factors will be considered in the following sections.

#### EFFECT OF HIGH TEMPERATURES UPON THE GERMINATION OF IMPERMEABLE CLOVER AND ALFALFA SEEDS

DRY HEATING.—Storing seeds for 6 months in a dry atmosphere at 45° C. slightly increased the subsequent germination of previously impermeable alfalfa seeds, but had no effect upon impermeable seeds of red clover or sweet clover. These results differ from results obtained by Hiltner (11). This author found that drying red-clover seed for eight days at 35° slightly increased both the percentage which remained hard after a 10-day germination test and the percentage which softened but did not germinate.

Heating at 50° C. for 21 hours had no effect upon the softening or germination of impermeable seeds of red clover or sweet clover when later subjected to a germination test.

HEATING IN WET BLOTTERS.—Seeds which remained hard after a 6-day germination test at 24° C. were subjected in the wet blotters to a temperature of 36° for the following seven days, during which time duplicate lots remained at 24°. Table VII shows the average percentages which germinated, and which softened but did not germinate.

TABLE VII.—Softening and germination of impermeable clover and alfalfa seeds at 24 and at 36° C.

Kind of seed.	Number of lots.	Number of seeds tested at—		Average percentage of germination at—		Average percentage which softened but did not germinate at—	
		24° C.	36° C.	24° C.	36° C.	24° C.	36° C.
Red clover.....	60	3, 220	3, 227	0. 48	1. 53	0. 09	0. 34
Alsike clover.....	15	1, 089	1, 068	1. 07	6. 00	. 15	. 99
White clover.....	11	826	820	. 41	. 51	0	1. 34
Sweet clover.....	17	1, 604	1, 607	. 94	. 94	. 06	. 45
Alfalfa.....	18	519	516	6. 31	10. 14	0	. 22
Crimson clover.....	2	48	56	1. 92	18. 27	0	. 96

1. A larger percentage of the seeds softened at 36° than at 24° C. The differences were small with red-clover, white-clover, and sweet-clover seed, somewhat larger with alsike-clover and alfalfa seed, and over 16 per cent with crimson-clover seed.

2. With the exception of white-clover seed at 36° C. nearly all the seeds which softened germinated. However, a somewhat larger proportion of the seeds which softened failed to germinate at 36° than at 24°.

In view of the very slight effect of heating at 36° C. for seven days in wet blotters, it hardly seems possible that soaking clover seeds over night in water at 34° before planting can bring about the germination of impermeable seeds as suggested recently by Müller (13).

#### EFFECT OF FREEZING TEMPERATURES ON THE GERMINATION OF IMPERMEABLE LEGUMINOUS SEEDS

One instance has already been mentioned in which impermeable sweet clover seeds previously lying dormant in the soil produced seedlings after a few days of freezing weather. (See Table VI.) A series of experiments was begun late in December, 1909, to test further the effect of freezing temperatures on the subsequent germination of impermeable leguminous seeds. The seeds used had lain in water without softening for 1½ to 5 months previous to the beginning of the experiment.

Different lots of the seeds were tested in a germinating chamber at about 20° C., in very moist soil in drinking glasses which were covered with black paper to exclude the light, and in soil in greenhouse flats. All of the seeds were subjected to freezing temperatures either before or during the germination tests, as follows:

The seeds which were tested in a germinating chamber at 20° C. were previously subjected, either dry or in small vials of water, to a temperature of about -10° C. One lot of each sample was given this treatment for 9 days and another lot during two periods of 9 days and 16 days, respectively, with an intervening period of a few hours in the laboratory at ordinary room temperature. As one exposure to this temperature had practically the same effect as two such exposures, only the average results of the tests of the two lots are herein considered.

TABLE VIII.—*Germination of impermeable leguminous seeds with and without freezing*

Kind of seed.	Test No.	Germination, impermeable seeds, and dead seeds.	Check test in chamber.	Tested in chamber after freezing in dry condition.	Tested in chamber after freezing in water.	Tested in soil on window ledge; frozen during test.	Tested in soil in greenhouse flats; frozen during test.
Red clover.....	85262	Percentage of germination.	6	8	26	21	.....
		Percentage of impermeable seeds.	93	92	72	65	.....
		Percentage of dead seeds.	1	0	2	14	.....
	85272	Percentage of germination.	5	13	9	32	38
		Percentage of impermeable seeds.	94	85	85	57	.....
		Percentage of dead seeds.	1	2	6	11	.....
Alsike clover.....	84087	Percentage of germination.	12	14	10	17	50
		Percentage of impermeable seeds.	87	82	80	72	.....
		Percentage of dead seeds.	1	4	10	11	.....
White clover.....	85192	Percentage of germination.	3	10	11	10	30
		Percentage of impermeable seeds.	96	89	88	81	.....
		Percentage of dead seeds.	1	1	1	9	.....
Sweet clover.....	78539	Percentage of germination.	2	1	1	4	74
		Percentage of impermeable seeds.	97	99	98	78	.....
		Percentage of dead seeds.	1	0	1	18	.....
Alfalfa.....	78479	Percentage of germination.	4	20	14	6	56
		Percentage of impermeable seeds.	95	80	84	84	.....
		Percentage of dead seeds.	1	0	2	10	.....
Black locust.....		Percentage of germination.	1	14	7	2	8
		Percentage of impermeable seeds.	99	84	90	96	.....
		Percentage of dead seeds.	0	2	3	2	.....

The seeds in the drinking glasses were so placed that they could be examined through the glass. The drinking glasses were kept on a shaded window ledge outside the laboratory during two periods of 10 days and 14 days, respectively. Each of these periods included several cold days during which the soil became frozen clear to the bottoms of the glasses. During an intervening period of 9 days and again after the second period on the window ledge they were kept in the laboratory at ordinary room temperature.

The greenhouse flats were kept outdoors during the entire experiment. The soil in them was alternately frozen and thawed at intervals during the first two or three months.

Check tests were made in a germinating chamber at about 20° C. without previous treatment of the seeds.

All of the tests were continued until March 24, 1910, and those in the greenhouse flats until May 7, 1910.

Table VIII shows the percentages of the seeds which germinated or produced seedlings in the different tests, the percentages which remained impermeable, and the percentages which softened but did not germinate in all of the tests except those which were conducted in greenhouse flats.

1. Subjection to a freezing temperature previous to the germination test slightly increased the percentages of the impermeable red-clover, white-clover, alfalfa, and black-locust seeds which germinated. In some cases, especially when the seeds had been frozen in water, this treatment increased also the percentages which softened but did not germinate. This latter effect is partly the result of the fact that a part of the seeds which softened after the first period of freezing were killed by the second freezing.

The impermeable sweet-clover seeds were wholly unaffected by this treatment.

2. The effect of freezing the impermeable seeds in soil in drinking glasses was similar to but greater than the effect of subjecting them to a freezing temperature previous to a germination test in a germinating chamber. Nearly all of the seeds which softened following each period upon the window ledge softened during the first few succeeding days in the laboratory.

3. The percentages of the impermeable seeds of the clovers and alfalfa which produced seedlings in the greenhouse flats with frequent freezing and thawing were much greater than the percentages which germinated in the germinating chamber or in the drinking glasses. This was particularly noticeable with the sweet-clover and alfalfa seeds. Nearly all of the seedlings appeared during warm days immediately following freezing weather.

Only 8 per cent of the impermeable black-locust seeds produced seedlings, and these few seedlings appeared after settled warm weather had begun late in March.



## EFFECT OF ALTERNATIONS OF TEMPERATURE ON THE GERMINATION OF IMPERMEABLE CLOVER AND ALFALFA SEEDS

Seeds remaining impermeable after from 4 months to over 12 months in wet blotters were kept in a chamber at room temperature for 49 days. The seeds were then kept for 50 days in chambers which, during a large part of the time, were heated daily to about 30° C. and then allowed to cool slowly to room temperature. Finally the seeds were again kept in chambers at room temperature for 51 days. Table IX summarizes the germination records for these three successive periods.

TABLE IX.—*Germination of impermeable clover and alfalfa seeds during successive periods of similar length with different temperature conditions*

Kind of seed.	Number of impermeable seeds used.	Approximate percentages of germination during—		
		49 days at room temperature.	50 days with frequent heating to 30° C.	51 days at room temperature.
Red clover.....	2,382	3	7	I
Alsike clover.....	118	0	2	I
White clover.....	270	I	2	I
Sweet clover.....	637	2	I	I
Alfalfa.....	230	25	21	7

The use of the alternating temperatures increased very slightly the germination of red-clover, alsike-clover, and white-clover seed, but did not influence the germination of sweet-clover and alfalfa seed. In no case did more than 11 per cent of the impermeable clover seeds germinate in the five months included in the three periods of observation.

Clover seeds which remained impermeable after various lengths of time in wet blotters were tested for germination at 1° C. in an ice box averaging about 10°, at 20°, at 30°, and with daily alternations between each two of these temperatures. When alternations of temperature were used, the seeds were kept in the chamber at the warmer temperature for about seven hours of each day and in the chamber at the cooler temperature the remainder of the day. In each test a succession of several temperature conditions was used, each condition being maintained for several days.

Figure 4 shows graphically the results of a series of tests of red-clover seeds from a single original lot which remained impermeable after four days' soaking in water. During these tests many seeds softened and remained for several days after softening at a temperature too cold for their germination. These softened seeds, as well as those which softened at warmer temperatures, invariably germinated later if subjected to a temperature favorable for germination. In order to emphasize the effects of the different temperatures, figure 4 shows only the rates of softening of the seeds without regard to their immediate germination.

During the first few days of the test from 5 to 7 per cent of the seeds softened under each temperature condition, showing that not all the easily permeable seeds had been removed by the previous soaking. After the first seven days the rate of softening varied according to the temperature conditions of the different tests.

1. When the alternation of temperatures from the ice box to 30° C. was used after a period of incubation in the ice box the seed softened rapidly for a few days, but the rate of softening diminished greatly within a week and soon fell off almost entirely.

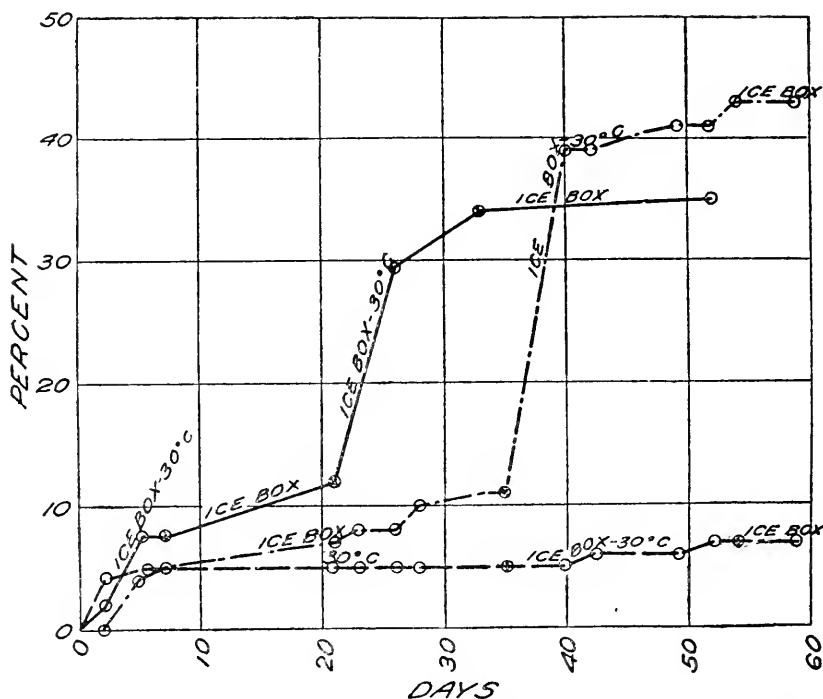


FIG. 4.—Curves showing the rate of softening of impermeable red-clover seeds under different temperature conditions.

2. While 32 per cent of the seeds softened when the alternation of temperatures was used after 35 days in the ice box, only 22 per cent softened with the same alternation after 14 days in the ice box.

3. When the alternation of temperatures followed 35 days at 30°, only 2 per cent softened in 19 days.

Results similar to those just outlined have been obtained with red-clover, alsike-clover, white-clover, and sweet-clover seeds which had previously remained impermeable in wet blotters for from two years to five years. The alternation from 1° to 30° C. has been as effective as the alternation from 10° to 30°. The alternation from 1° or 10° to 20° has had somewhat less effect. In any case the effectiveness of an alternation

has always depended upon a previous incubation of the seeds at a cool temperature. Usually the longer the previous period of cool incubation the greater has been the effect of the alternation. Alternations from  $1^{\circ}$  to  $10^{\circ}$  or from  $20^{\circ}$  to  $30^{\circ}$  had little or no effect.

In some cases more than 90 per cent of seeds which had previously remained impermeable in wet blotters for several years have softened and germinated in a few weeks with a favorable succession of constant cool temperatures and alternations of temperature.

The work outlined in this section is of special interest in connection with the following sections. The results here presented would lead one to expect that impermeable clover seeds would lie in the soil without change during either steady cold weather or constantly warm weather and in the fall, when an alternation of warm days and cool nights follows the hot summer months, but that many of them would germinate and produce seedlings at the beginning of the growing season in the spring when a similar alternation follows months of cold winter weather. These results also show that subjection to freezing temperatures is not necessary in order to prepare the majority of impermeable clover seeds for rapid germination. A temperature of about  $10^{\circ}$  C. does quite as well as  $1^{\circ}$ , and either of these temperatures is much more effective under favorable conditions than freezing temperatures under conditions which are less favorable. (See p. 776-778 and Table VIII.)

#### GERMINATION OF SEEDS AFTER PASSING THE WINTER ON OR UNDER THE PARENT PLANTS IN THE FIELD

Seeds which had passed one winter on or under the parent plants in the field were gathered the following spring after warm weather had begun. The germinating capacity of these seeds and the percentages which were impermeable were determined and compared with the germinating capacity and percentage of impermeable seeds of lots of seed which were gathered from the same stands of plants the preceding fall and stored in the laboratory during the winter.

The red-clover seeds which were gathered in the spring consisted of eight lots from heads which remained intact upon the parent plants and were several inches above the ground, and eight other lots from heads which were embedded in the mud.

The alsike-clover, sweet-clover, and yellow-trefoil seeds gathered in the spring were all embedded in the mud. A large number both of these and of the red-clover seeds which were embedded in the mud had germinated and produced a dense growth of strong green seedlings before they were gathered. Many red-clover seeds had softened, and a few had germinated even in the heads which were several inches above the soil. About half an inch of soil was taken up with the seedlings which were growing in the soil, and carefully worked over to re-

move all the seeds. All seeds which had softened and looked healthy were counted as germinated.

The red-clover seeds which were impermeable when removed from the soil or from the seed heads were subjected to a germination test for one month in a chamber at about 20° C. The seeds which germinated in the germinating chamber were included with those which germinated in the soil in determining the germinating capacity.

All the alfalfa seeds gathered in the spring were from pods which remained upon the straw a foot or more above the soil. Ninety per cent or more of the seeds in these pods were brown and dead, and some were partly disintegrated. The remaining 10 per cent or less were bright, plump, and yellow. These were retained for the germination test. All alfalfa seeds which could be found in the surface soil under the plants were dead.

There were included in the examination 9,723 red-clover seeds, 575 alsike-clover seeds, 412 sweet-clover seeds, 200 alfalfa seeds, and 99 yellow-trefoil seeds, which were gathered in the spring.

Table X gives the results of the investigations.

TABLE X.—Germination of leguminous seeds after passing the winter on or under the parent plants in the field compared with the germination of seeds harvested the previous fall

Kind of seed.	Number of lots.	Season in which gathered.	Average percentage of—			Calculated average percentage of the seeds previously impermeable which became permeable.
			Germination.	Impermeable seeds.	Dead seeds.	
Red clover.....	8	Fall, 1909.....	8	88	4	1
Do.....	8	March, 1910 <sup>a</sup> ..	56	33	1	63
Do.....	8	March, 1910 <sup>b</sup> ..	37	61	2	30
Alsike clover.....	1	Fall, 1912.....	15	84	1	-8
Do.....	1	April, 1913 <sup>c</sup> ..	59	37	4	52
Sweet clover.....	1	Fall, 1912.....	10	90	0	-2
Do.....	1	April, 1913 <sup>c</sup> ..	63	36	1	59
Alfalfa.....	1	Fall, 1913.....	76	22	2	.....
Do.....	1	April, 1914 <sup>d</sup> ..	3	97	0	.....
Yellow trefoil.....	1	April, 1913 <sup>c</sup> ..	64	36	0	.....

<sup>a</sup> From heads embedded in the soil; germination reported includes 1 month in chamber.

<sup>b</sup> From heads on the straw above the soil; germination reported includes 1 month in chamber.

<sup>c</sup> Germination in the field only.

<sup>d</sup> From dry heads well above the soil; germination in chamber.

1. From 52 to 63 per cent of the clover and yellow-trefoil seeds which were impermeable in the fall softened after passing the winter in the soil. Of the impermeable red-clover seeds which were in heads several inches above the soil 30 per cent softened. In the meantime only 1 per cent of the impermeable red-clover seeds which were stored in the laboratory over the winter became permeable, and the percentage of alsike-clover

and sweet-clover seeds which were impermeable increased under dry storage in the laboratory. Nearly all of the seeds which softened after wintering in the soil or on the plants germinated.<sup>1</sup>

2. Only those alfalfa seeds which remained impermeable survived the winter on the plants. Only 3 per cent of those seeds gathered in the spring germinated and 97 per cent were impermeable. Of those gathered in the fall 76 per cent germinated and 22 per cent were impermeable, only 2 per cent being dead.

Additional tests were made upon self-sowed seed in February and March, 1916, taking advantage of the effect of a favorable alternation of temperatures upon the softening of the seeds (see p. 779-781), as follows:

One lot of self-sowed red-clover seed and two lots of self-sowed sweet-clover seed, with the soil in which they were embedded, were gathered on February 29 and immediately placed in an ice box in which the temperature was constantly somewhat below 10° C. During the next few days the seeds were separated from the soil without allowing them to become dry at any time. Both before and after removing them from the soil they were daily alternated between the ice box and the germinating chamber at 30°.

Many seeds had produced seedlings, and others had softened but had not germinated in the field. Many of the seed which were impermeable when taken into the laboratory softened in the next four days. After the fourth day there was very little change during the following three weeks, although the seeds were incubated in the ice box for nine days and then again alternated between the ice box and the chamber at 30° C.

The numbers of seeds and seedlings recovered from the soil were as follows: Of red clover, 4,610; of the two lots of sweet clover, 1,508 and 980, respectively. By the end of the fourth day after collecting the seeds, 86, 54, and 66 per cent of these different lots had softened either in the field or in the laboratory. If it be assumed that 90 per cent of these seeds were impermeable the preceding fall, it can be calculated that 84, 49, and 62 per cent of the impermeable seeds softened.

Besides the leguminous seeds already considered, eight lots of okra seeds were gathered in April, 1913, after passing the winter in the field. The great majority of these seeds were dead, but the percentage of dead seeds varied according to the previous exposure, being 69 per cent of the seeds in closed pods on the ground, 91 per cent of the seeds in closed pods on the stalks, 95 per cent of the seeds in opened pods on the stalks, 99 per cent of the seeds in opened pods on the ground, and all of the shelled seeds lying loose on the ground. Of the seeds which softened without clipping none germinated except of those which had been wintered in closed pods on the ground, where they had the full protection

<sup>1</sup> In this connection Hume's observations on sweet clover in South Dakota are interesting (12). Unhulled sweet-clover seed was sowed in August, 1911 and in 1912. Only a few seedlings were produced the year the seeds were sowed, but in each case a good stand of sweet-clover plants came up the following spring.

of the pod and a part of the time the protection of a snow cover. Of seeds which were gathered from the same cultivated rows the preceding fall and stored in the laboratory, 23 per cent germinated, 71 per cent were impermeable, and only 6 per cent were dead.

PRODUCTION OF SEEDLINGS BY IMPERMEABLE SEEDS DURING ONE YEAR  
IN GREENHOUSE FLATS WITH FREEZING AND THAWING

Seeds of a number of lots of red clover, alsike clover, white clover, sweet clover, crimson clover, alfalfa, and okra were sowed in rows in greenhouse flats on March 18, 1911, and the tests were continued for 12 months.

From November 18 to December 11 and again from December 20 to January 25 the flats were outdoors. Each of these periods included some very cold days during which the soil in the flats became thoroughly frozen.

During the eight months previous to November 18, during the nine days between the two outdoor periods, and again from the end of the second period out of doors on January 25 to the end of the experiment on March 19, 1912, the flats were kept in a greenhouse.

At the end of the first 11 days with the clovers and alfalfa and at the end of the first 22 days with okra the percentages of seedling production in the greenhouse flats were approximately the same as the percentages of germination in a germinating chamber in, respectively, 4 and 10 days. All seedlings which appeared after the first 11 or 22 days were considered as being produced by impermeable seeds.

Although the experiment was continued in the greenhouse for nearly two months after the end of the second period of freezing, very few seedlings were produced after the first week of that time.

At the end of the experiment the soil was dried, broken up, and sifted through sieves of the proper sizes, and as many as possible of the seeds which still remained impermeable were recovered.

TABLE XI.—*Production of seedlings by impermeable clover, alfalfa, and okra seeds when submitted to freezing and thawing*

Kind of seed.	Number of impermeable seeds found in chamber test.	Calculated percentages of the impermeable seeds which produced seedlings.		Percentages of the impermeable seeds—	
		In eight months before freezing.	After freezing.	Recovered from the soil.	Decayed or lost.
Red clover . . . . .	100	20	28	7	45
Alsike clover . . . . .	52	4	23	9	64
White clover . . . . .	136	3	15	21	61
Sweet clover . . . . .	448	5	61	11	23
Alfalfa . . . . .	162	37	4	2	57
Crimson clover . . . . .	44	50	0	0	50
Okra . . . . .	340	38	1	12	49

Table XI shows the calculated percentages of the impermeable seeds which produced seedlings before and after the freezing of the soil, the percentages which were recovered from the soil after the experiment, and the percentages which decayed or were lost.

1. From one-third to one-half of the impermeable seeds of alfalfa, crimson clover, and okra produced seedlings during the first eight months, while the flats remained in the greenhouse, but no crimson-clover seedlings and only a few seedlings of the other species appeared after the periods of freezing.

2. A small percentage of the impermeable seeds of red clover, alsike clover, white clover, and sweet clover produced seedlings in the first eight months, and a considerably larger percentage after the freezing of the soil. The seedling production after the freezing of the soil was particularly large (61 per cent) with sweet clover.

3. Only small percentages of the seeds were recovered from the soil at the close of the experiment. Approximately one-fourth of the sweet-clover seeds and approximately one-half of the seeds of the other species of plants were unaccounted for. While undoubtedly a few of these were lost, the majority of them must have softened and decayed during the experiment. The surface of the soil was at times crusted, and toward the end of the experiment much of it was thickly overgrown with moss. These conditions probably prevented some seedlings from reaching the surface even when the seeds germinated normally.

#### PRODUCTION OF LEGUMINOUS SEEDLINGS IN THE FIELD COMPARED WITH GERMINATION IN A GERMINATING CHAMBER

In May, 1912, and again in May, 1913, field tests were conducted in comparison with chamber germination tests. The soil used was a rich sandy loam which held water well and was easily pierced by the young seedlings.

Table XII shows the percentages of impermeable seeds (determined in chamber test), the percentages of chamber germination in from four to eight days, and the percentages of seedling production in from ten to twenty days.

1. In the tests of 1912 the percentages of germination were greater than the percentages of seedling production in the field. However, those lots which contained small percentages of impermeable seeds produced much larger percentages of seedlings than those lots which contained large percentages of impermeable seeds.

2. There was a striking difference between those stands of plants secured from lots of impermeable seed and those stands secured from lots of seed but few of which were impermeable. Plate CVI shows this difference for alsike-clover and white-clover plants produced in 1912. The photographs were taken about four months after the seeds were

planted. In each photograph the two side rows were from one lot of seed over 90 per cent of which germinated in the chamber (Table XII, No. 146785 and 145571), and the middle row, with only a few scattered plants, was from a lot of seed over 90 per cent of which was impermeable (Table XII, No. 140624 and 140670). The same number of seeds was planted in each row and both lots of each kind of seed were planted on the same day.

TABLE XII.—Seedling production by leguminous seeds in the field compared with germination in a germinating chamber

Kind of seed and year in which test was made.	Test No.	Percentage of impermeable seeds according to chamber test.	Percentage of germination in chamber in from four to eight days.	Percentage of seedlings in field in from ten to twenty days.
1912.				
Red clover.....	83843	54	46	38
	85371	65	34	29
	85454-0	38	62	49
	85454-1	72	28	28
	124135	81	18	8
	146458	2	94	67
	146582	1	95	59
	140624	94	4	3
	140785	3	96	80
	140670	96	4	3
Alsike clover.....	145571	5	94	69
White clover.....	29406	97	3	5
	123687	3	45	28
Sweet clover.....	145394	20	80	52
	146673	0	97	65
1913.				
Red clover.....	82955	78	22	32
	149080	4	96	93
Alsike clover.....	140624	94	3	3
	148714	9	90	80
White clover.....	140670	95	3	7
	148502	4	94	83
Sweet clover.....	78539	87	6	9
	119982	16	67	64
Alfalfa.....	G I.....	71	25	44
	149305	3	96	84
Hairy vetch.....	A I.....	5	85	73

3. Figure 5 represents graphically for each lot of seed used in 1913 the percentage of germination in 8 days, the percentage of seedlings produced in the field in from 16 to 18 days, and the sum of percentage of germination and percentage of impermeable seeds. The space between the line at the top of the figure (germination plus impermeable seeds) and the lowest line in the figure (chamber germination) represents the percentage of impermeable seeds in each lot. The line representing chamber germination crosses the line representing seedling production at a point which corresponds to 60 per cent of germination, with nearly all



of the other 40 per cent of the seeds impermeable. When more than 40 per cent of the seeds in a lot were impermeable the percentage of seedling production was greater than the percentage of chamber germination; when less than 40 per cent were impermeable, chamber germination exceeded seedling production. We see here undoubtedly the combined

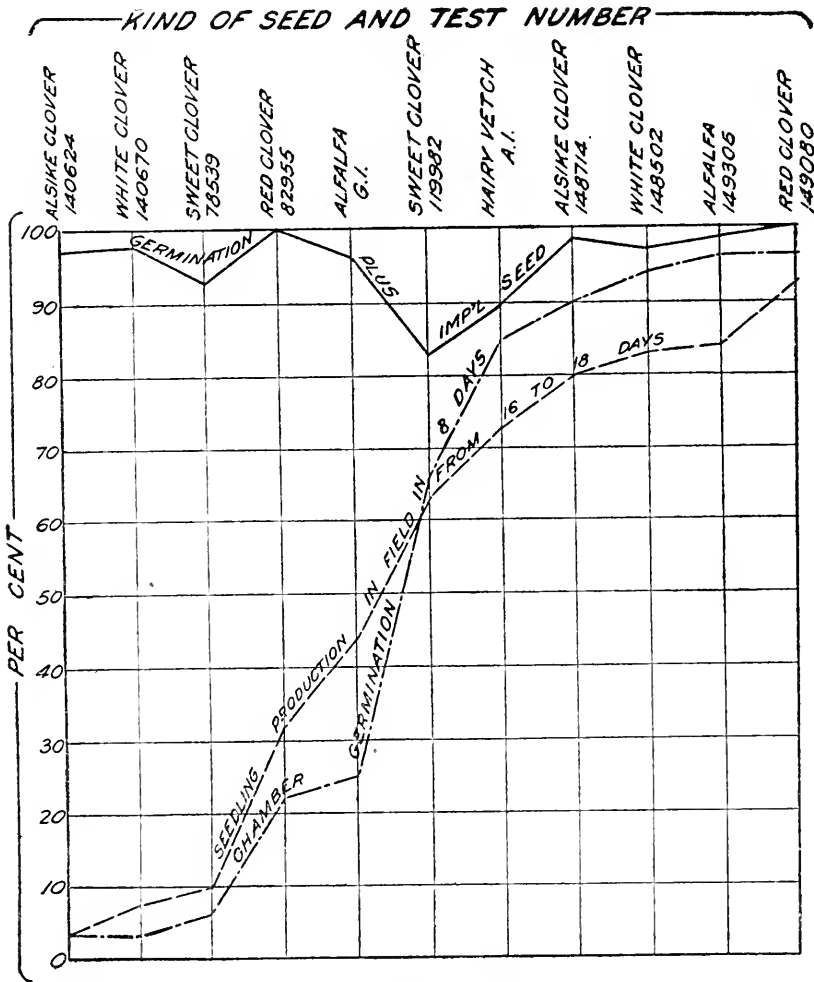


FIG. 5.—Curves of the seedling production in the field in 16 to 18 days and of the germination in chamber in 8 days.

effect of two separate conditions: First, a larger percentage of the impermeable seeds germinated in the field than in the chamber; second, some of the seeds which germinated in the soil did not produce seedlings which penetrated the overlying soil and were counted.

## FIELD TESTS AND CHAMBER GERMINATION TESTS CONTINUED FOR ONE YEAR

The field tests begun in May, 1913, were kept under observation for nearly 13 months. In making these tests 500 seeds of each lot were spaced 2 inches apart in rows 4 inches apart in well-prepared beds which had been steam sterilized to kill weed seeds.

Observations were made frequently throughout the summer and as late as the middle of October. The plants were occasionally thinned to prevent crowding and to facilitate the observations. Early in November the beds were covered with cheesecloth on wooden frames to protect them from contamination by other seeds.

No observations were made after the cheesecloth covers were put in place until January 2 and 3 at the close of a period of warm, rainy weather. At this time there were a large number of new seedlings in the beds which were planted with lots of seed containing large percentages of impermeable seeds and a few in beds which were planted with lots of seed containing small percentages of impermeable seeds. Many of these seedlings had been heaved out by preceding freezes and there were evidences that some seedlings had been destroyed by insects. All of the seedlings which appeared in January and were not otherwise destroyed were killed by subsequent freezing and thawing.

By the 23d of March healthy clover seedlings of all kinds had appeared in abundance in protected places in the vicinity of the sterilized beds. On this day the cheesecloth covers were permanently removed.

TABLE XIII.—Seedling production by impermeable clover and alfalfa seeds in the field in 12½ months compared with germination in a germinating chamber

Kind of seed.	Calculated percentages of impermeable seed which produced seedlings in field.					Percentages of impermeable seeds which germinated in chamber at room temperature in 12½ months.
	In first 16 to 18 days.	During season planted.	Following winter. <sup>a</sup>	Following spring. <sup>b</sup>	Total in 12½ months.	
Red clover.....	13	17	18	39	74	31
Alsike clover.....	0	3	10	45	58	5
White clover.....	4	7	<sup>c</sup> 4	<sup>c</sup> 39	50	16
Sweet clover.....	4	8	17	27	52	7
Alfalfa.....	27	74	1	.....	75	66

<sup>a</sup> These seedlings appearing in midwinter were killed by later freezing.

<sup>b</sup> Produced 1914 stand of plants.

<sup>c</sup> About three-fifths of the white-clover bed became so covered with the growth of plants produced in 1913 that observations the following winter and spring had to be confined to the other two-fifths of the bed. From these observations the percentages for the whole bed were calculated.

No new seedlings appeared during February, which was very cold, New growth began late in March and new seedlings appeared in increasing numbers from this time to about the middle of April and more slowly

thereafter until about the middle of May. Very few appeared later than May 20. Wherever these seedlings were densely shaded by healthy plants of the preceding season they grew tall and slender at first and ultimately disappeared.

Table XIII shows the calculated percentages of the impermeable seeds of lots originally containing large percentages of impermeable seeds which produced seedlings during the first 16 to 18 days, the first spring and summer, during the following winter, during the following spring, and the total percentages of impermeable seeds which produced seedlings in the field and which germinated in the chamber in 12½ months.

1. From 5 to 16 per cent of the impermeable seeds of alsike clover, white clover, and sweet clover and 31 per cent of the impermeable red-clover seeds germinated in the chamber in 12½ months. During the same time 74 per cent of the impermeable red-clover seeds and from 50 to 58 per cent of the impermeable seeds of the other kinds of clover produced seedlings in the field. Of these seedlings from one-twentieth to one-fourth appeared during the season in which the seeds were planted, from one-twelfth to one-third appeared during the following winter, but were killed before the winter was over, and the remainder, representing from one-fourth to one-half of the whole number of impermeable seeds planted, appeared in the spring of 1914 and produced the 1914 stand of plants.

2. Of the impermeable alfalfa seeds 74 per cent produced seedlings in the field during the first season and 1 per cent the following winter, while only 66 per cent germinated in the chamber in 12½ months. No observations were made on this lot in the field in the spring of 1914.

3. From one-fourth to one-half of the impermeable seeds of the different kinds either remained impermeable in the soil or softened and died.

Figure 6 shows graphically the percentages of seedling production in the field and of germination in chamber in 12½ months, and the sum of percentages of germination and of impermeable seeds according to chamber test. The different lots of seed are represented in the same order here as in figure 5, and the curve at the top of the figure is the same as occurs at the top of figure 5.

At the end of a year the seedling production by all the lots which contained large percentages of impermeable seeds had surpassed the germination in the chamber by an amount roughly proportional to the percentages of impermeable seeds which they contained. With all the lots which contained small percentages of impermeable seeds except sweet clover 119982 the percentages of seedling production in the field, even after 12½ months, were less than the percentages of chamber germination in eight days.

SUMMARY OF SEEDLING PRODUCTION IN SOIL BY IMPERMEABLE CLOVER AND ALFALFA SEEDS

The results of a number of separate experiments on seedling production by impermeable seeds have been discussed in the preceding pages. The different series of tests were made at different times and with different lots of seed. Although this fact makes a direct comparison of the

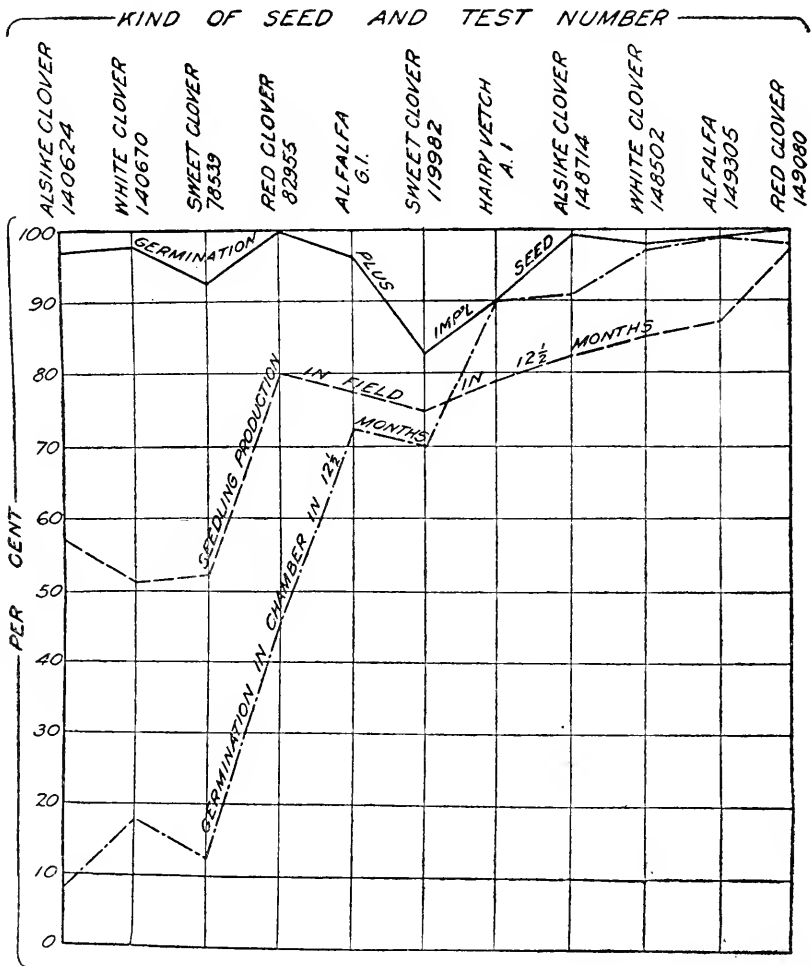


FIG. 6.—Curves of the seedling production in the field and of the germination in chamber in 12½ months.

results of the different series of tests impossible, some important considerations are emphasized by grouping the different series together, as in Table XIV. The results of check tests conducted in germinating chambers are included if such check tests were made; also the percentages of chamber germination of impermeable seeds in one year taken from Table III.

TABLE XIV.—Production of seedlings by impermeable seeds; assembled results of different experiments

Place and description of test.	Duration of test.	Average percentage of the impermeable seeds which germinated or produced seedlings.				
		Red clover.	Alsike clover.	White clover.	Sweet clover.	Alfalfa. <sup>a</sup>
Greenhouse (p. 774).....	3 months...	16	.....	5	5	38
Check in germination chamber, room temperature.....	do.....	5	.....	3	2	8
Cold frame (Table VI).....	6 months...	14	.....	.....	14	<sup>b</sup> 80
Cold frame, with freezing (Table VIII)	4 months...	38	50	30	74	56
Check in germination chamber, room temperature.....	3 months...	5	12	3	2	4
Seedlings produced in spring from seeds wintered in field (Table X).	.....	<sup>c</sup> 63	<sup>d</sup> 52	.....	<sup>d</sup> 59	.....
Seedlings produced in spring from seeds wintered in field (p. 23).	.....	<sup>c</sup> 84	.....	.....	{ <sup>d</sup> 49 <sup>d</sup> 62 }	.....
Cold frame and greenhouse with winter freezing (Table XI).	1 year.....	<sup>d</sup> 48	<sup>d</sup> 27	<sup>d</sup> 18	<sup>d</sup> 66	<sup>d</sup> 41
Field, in sterilized beds (Table XIII).	do.....	<sup>d</sup> 74	<sup>d</sup> 58	<sup>d</sup> 50	<sup>d</sup> 52	<sup>d</sup> 75
Check in germination chamber, room temperature.	do.....	31	5	16	7	66
Germination chamber; average of several commercial lots (Table III)	do.....	27	21	13	21	70

<sup>a</sup> When alfalfa seeds were in a frozen medium during any part of the experiment, nearly all seedlings were produced before freezing occurred.  
<sup>b</sup> In 2 months.  
<sup>c</sup> Calculated from heads embedded in the soil.  
<sup>d</sup> Calculated.

1. In each series of tests a large proportion of the impermeable alfalfa seeds produced seedlings in the soil. It should be remarked that in each series of tests nearly all of the seedlings produced from impermeable alfalfa seeds appeared during the first month or two of the experiment and that very few alfalfa seedlings appeared after the freezing of the soil except when the seeds were too cold to germinate from the beginning of the test period until after the freezing had occurred. (See Table VIII and accompanying text.) An examination of alfalfa seeds which had passed one winter under the parent plants shows that continued severe freezing and thawing in wet soil will soften and kill practically all (p. 783).

2. With each of the various kinds of clover the percentage of seedling production was small when no freezing of the soil occurred during the experiment.<sup>1</sup> Seedling production from impermeable clover seeds was greatly increased by the freezing of the soil and was greatest (except with sweet clover) in the series of field tests which were continued for a year.

In every series of experiments in which check tests were made in the germination chamber the average percentage of seedling production both of alfalfa and of the clovers was greater than the average percentage of

<sup>1</sup> In a few cases with individual lots of seed of alsike clover and white clover, a large percentage of the impermeable seeds produced seedlings in a short time in soil in a moderately warm greenhouse. These cases were so rare as to be almost negligible.

chamber germination. The differences were insignificant in some cases, but were very large in the field tests of the clovers continued for one year.

In this connection the effect of temperatures a few degrees above freezing should be emphasized. The laboratory tests in which certain alternations of temperature were used following cool constant temperatures show conclusively that actual freezing is not necessary in order to cause the subsequent softening and germination of many impermeable clover seeds (p. 781). Moreover, in many cases larger percentages of the impermeable seeds softened and germinated in these tests than in any of the tests with seeds which had passed the cold winter months in the soil under the parent plants (p. 781-784). These facts indicate that impermeable clover seeds would germinate as well if sowed several weeks before the beginning of warm weather in the spring as if sowed the preceding fall. In addition, spring sowing would avoid the danger of winter-killing softened seeds or young seedlings.

#### USE OF IMPERMEABLE SEEDS

The value to the farmer of the impermeable seeds occurring in any lot of seed will vary according to the kind of seed, the germinating capacity, the percentage of impermeable seeds in the lot of seed under consideration, the age of the seed, and the time of sowing the seed.

Impermeable alfalfa seed sowed late in the spring is of more value to the crop than impermeable sweet-clover seed sowed at the same time.

If the percentage of impermeable seed in a given lot is small (10 per cent or less) and the rest of the lot consists of strong, germinable seeds, the impermeable seeds are of little importance both because of their fewness in comparison with the seeds which germinate readily and because of the varying quantities of seed which are sowed according to common practice. It is when the impermeable seeds constitute a large percentage of the seed in a given lot that their real value becomes a question of agricultural importance.

In seed that is several years old the viability of the permeable seeds may have become so low that the impermeable seeds, which lose their vitality more slowly, are relatively much more important than in lots of fresh, new seeds.

Impermeable clover seed sowed early in the spring is of more value than the same seed sowed later, when the weather has become settled and warm.

The following general rules, based upon the experimental results and upon the considerations just outlined, are suggested as guides in agricultural practice with the plants investigated.

Assuming that all seeds have been tested for germinating capacity and percentage of impermeable seeds, calculate the amount of seed to sow as specified below.

I. RED CLOVER, ALSIKE CLOVER, WHITE CLOVER, AND WHITE SWEET CLOVER

A. When seed is to be sowed in the late spring or summer.—Consider one-tenth of the impermeable seed as good. Add one-tenth of the percentage of impermeable seed to the percentage of germination. Calculate from this sum the quantity of seed of the given lot necessary to give the desired quantity of good germinable seed. For example: It is desired to sow per acre the equivalent of 15 pounds of viable seed none of which is impermeable. Fifty per cent of the lot of seed to be used germinates and 40 per cent is impermeable. To 50 per cent add one-tenth of 40 per cent, or 4 per cent. Consider 54 per cent as good. Then divide 15 by 0.54. The quotient, or 27.8, is the number of pounds of seed to sow per acre. In the form of an equation we have the following statement:

$$\frac{\text{Number of pounds good seed desired per acre}}{\text{Percentage of germination} + \frac{1}{10} \text{ the percentage of impermeable seed}} = \left\{ \begin{array}{l} \text{Number of} \\ \text{pounds} \\ \text{to sow} \\ \text{per acre,} \end{array} \right.$$

$$\text{or } \frac{15}{\frac{0.50 + 0.40}{10}} = 27.8$$

The impermeable seeds remaining in the ground will constitute a reserve which, under favorable conditions in a cold climate, will improve any thin areas in the stand the following spring. This, however, should not be counted upon, as spots not occupied by desirable plants before the second growing season will almost certainly be appropriated by more rapidly growing weeds unless the field is unusually free from weed seeds.

B. When seeding in the late fall or winter or in the spring a month or so before the end of freezing weather.—Consider all of the impermeable seeds as good. Add the percentage of impermeable seeds to the percentage of germination. Calculate from the sum the quantity of seed to be used, as under A. For instance, in the example given under A add 40 per cent to 50 per cent, which gives a total of 90 per cent. Then divide 15 by 0.9. The quotient, 16.7, is the number of pounds to sow per acre. Probably not all the impermeable seeds will soften and produce seedlings, but the seedlings produced by them will be less liable to injury than the seedlings produced by permeable seeds which soften immediately, germinate on the first warm days, and may be killed by subsequent freezing.

C. When seeding in the spring after danger of severe frost but a month or more before the end of cool weather.—Consider two-thirds of the impermeable seeds as good and proceed as under A and B.

2. ALFALFA AND CRIMSON CLOVER

To the percentage of germination add two-thirds of the percentage of impermeable seeds and calculate the quantity of seed to be used as given under red clover. More than two-thirds of the impermeable seeds may germinate, but not soon enough to compete with weeds.

3. HAIRY VETCH

To the percentage of germination add one-half of the percentage of impermeable seeds as a basis for calculating the quantity of seed to be used. Proceed as under red clover.

4. OKRA

To the percentage of germination add one-fourth of the percentage of impermeable seeds as a basis for determining the quantity of seed to sow, and proceed as under red clover. More than one-fourth of the impermeable seed will probably germinate, but too late to contribute to a uniform stand.

## CONCLUSION

By "impermeable seeds" is meant those seeds all parts of whose seed coats are impermeable to water at temperatures favorable for germination.

It is impossible to distinguish between impermeable and permeable seeds except by testing their ability to absorb water at a temperature favorable for germination.

The production of impermeable seeds is particularly characteristic of the Leguminosae, but it occurs also in many other plant families.

Among the cultivated species which sometimes produce impermeable seeds are okra, hollyhock, alfalfa, atriplex, asparagus, morning-glory, canna, cherry tomato, and nearly all of the cultivated species of Leguminosae.

Impermeable seeds frequently retain their vitality for many years, sometimes for at least as many as 80 years.

Fresh impermeable seeds germinate promptly when the seed coat is broken or becomes permeable.

The viability of fresh impermeable seeds is frequently greater than the viability of fresh seeds of the same species which are permeable.

Seeds of the common clovers, alfalfa, and hairy vetch which are impermeable at the end of three to five years under laboratory conditions of storage retain their vitality apparently unimpaired up to that time. The viability of the permeable seeds in the same lots decreases slightly in the second and third year and more in subsequent years.

In dry storage nearly all impermeable alsike-clover, white-clover, and sweet-clover seeds remain impermeable until at least 2 or 3 years old. Impermeable red-clover seeds become permeable gradually in dry storage, but from one-third to two-thirds of them may still be impermeable after four years. The majority of impermeable alfalfa and hairy-vetch seeds become permeable before they are 2 years old. Okra seeds become less permeable as their age increases.

In wet blotters nearly all impermeable alfalfa, crimson-clover, hairy-vetch, and okra seeds soften and germinate in one year, though a very few may remain impermeable even after three or four years. Impermeable seeds of red clover, alsike clover, white clover, and sweet clover soften and germinate more slowly, but with no uniformity as to rate. All germinate within one year in some cases, while in other cases over 50 per cent are still impermeable after four years.

Impermeable clover seeds which were thoroughly matured before harvesting soften and germinate more slowly under conditions favorable for germination than do impermeable seeds of the same species which were less well matured; they also become permeable more slowly in dry storage.

Impermeable seeds become permeable more rapidly in wet blotters than in dry storage.



It is impossible to estimate even approximately in advance the proportion of the impermeable seeds in any given lot which will germinate in any given length of time under ordinary germination conditions.

A widely variable proportion of the impermeable seeds of alfalfa, crimson clover, and the larger seeded commercial species included in this investigation produce seedlings promptly in the soil under greenhouse conditions or in the open field in warm weather. Only in exceptional cases is this true of the impermeable seeds of the clovers, other than crimson clover.

The use of aqueous extracts from soil has no effect, and alternate wetting and drying of the seeds has but little effect on the germination of impermeable seeds.

Within ordinary limits neither the depth of planting nor the firmness of the soil affects the germination of impermeable clover and alfalfa seeds under greenhouse conditions. These factors may affect the stand secured by preventing some of the seedlings from reaching the surface.

Storing impermeable clover and alfalfa seeds at a temperature of 50° C. for one day or at 45° for six months has little or no effect upon their germinating capacity or permeability.

In wet blotters a temperature of 36° very slightly increases the softening of the impermeable seeds, but also kills some of the seeds.

Freezing, when wet, causes the subsequent germination of many impermeable seeds, but may kill some seeds which had previously softened.

Any constant temperature from 1° C. to 30° has little effect upon the softening of impermeable clover seeds.

Alternations of temperature have but little effect on the softening and germination of impermeable clover and alfalfa seeds if none of the temperatures used in the alternation is cooler than 20° C.

Alternations of temperature cause the softening and germination of many impermeable clover seeds when a temperature of 10° or cooler is used in alternation with a temperature of 20° or warmer. The effect of such an alternation of temperature is greatly increased by previously exposing the seeds to germination conditions at a cool temperature (10° C. or cooler), and is decreased by previously exposing the seeds to germination conditions at a warm temperature (30°).

Even under the most favorable conditions only a small proportion of impermeable red-clover, alsike-clover, white-clover, and white sweet-clover seeds produces seedlings promptly in the soil when sowed in warm weather.

Impermeable seeds of red clover, alsike clover, white clover, and white sweet clover will pass the winter in the soil in a freezing climate without injury. At least 50 or 60 per cent of them may be expected to germinate in the soil the following spring unless a part of them germinate during warm weather in the winter. If this occurs, the seedlings produced in the winter are liable to be killed by subsequent freezing.

A large proportion of impermeable alfalfa, crimson-clover, okra, and hairy-vetch seeds will germinate in the soil during the first few months after planting, some of them early enough to be of importance to the crop.

Nearly all alfalfa and okra seeds, even if they are impermeable in the fall, are killed when they pass the winter in the soil or on the plants out of doors in a freezing climate. A small proportion of the impermeable alfalfa seeds survive with their vitality uninjured. Some of the okra seeds remain impermeable during the winter, but the majority even of those which remain impermeable are killed by the winter's exposure.

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PLATE CVI

Fig. 1.—A row of alsike clover from impermeable seeds between two rows from permeable seeds.

Fig. 2.—A row of white clover from impermeable seeds between two rows from permeable seeds.





## MENDELISM OF SHORT EARS IN SHEEP

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Among the various features under observation in the experimental breeding carried on at this Station with sheep the "short" ear trait is a very clear example of a simple Mendelian unit factor.

Short ears as referred to here are of a distinctive type with nearly straight lines running from the base and forming an abrupt, sharp point. They are also somewhat thicker than the ordinary type of ear. The longest of these ears so far observed in a mature animal measure 7 cm. ( $2\frac{3}{4}$  inches). Length as a character therefore forms quite a distinctive contrast<sup>1</sup> between this type and that of Rambouillet ears, which measure about 11.5 cm. ( $4\frac{1}{2}$  inches); Southdowns, which measure about 9.5 cm. ( $3\frac{3}{4}$  inches); and Shropshires and native, which measure about 10 cm. (4 inches). In fact, all ordinary ear lengths observed among various breeds and types seem to run close around 10 cm. (4 inches) or over.

The experimental data given here are derived from one native ewe and her progeny, which number 15 head. This ewe was purchased from a neighboring farm with 19 other native ewes, none of which had short ears or short-ear offspring. The character of her dam is unknown, but her sire is known to have possessed long ears. She was therefore in all probability simplex as to the character of ear length. This short-ear ewe (No. 69) was bred to a Hampshire ram (No. 3) for three successive years, producing three female offspring, all short-eared. Two of these died, leaving only one (No. 127). As no  $F_1$  males of this type were available, she was used twice on a back cross with her sire (who was a pure long ear) and once on a similar back cross with No. 361 (also a pure long ear). From this cross three sets of twins were obtained with an equal number of short and long ears, which corresponds to the results expected from a back cross of a simplex to the recessive parent in a simple Mendelian unit character. She was later bred to her own son (No. 255), an offspring of this back cross, who showed the recessive trait. Being recessive, he should have been pure to long ears, and the cross on his dam should give similar results as the former matings of 127 with pure long-eared sires (No. 3 and 361). The actual result was one pair of twins, including one short-eared and one long-eared individual. This gave a total of four short-eared and four long-eared offspring from simplex  $\times$  recessive parents.<sup>2</sup>

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<sup>1</sup> No intermediate types either as to length, shape, or thickness have so far appeared.

<sup>2</sup> One mating was simplex  $\times$  extracted recessive.

The next type of mating was made with a short-eared male (No. 422), who was one of the pair of twins out of No. 127 by her son, and therefore her grandson. No. 422 was therefore simplex. He was bred to female 256, a simplex offspring of the first back cross (No. 3 × 127), and also to 127, who was simplex. These matings, being simplex × simplex, correspond to a mating of F<sub>1</sub> and should give the 3 to 1 ratio. Four offspring were obtained from these matings, three of which had short ears and one long, thus giving results again conforming numerically to theory as regards segregation of a simple Mendelian dominant character. The following diagram shows the various matings and their results. S indicates short ear; L, long ear.

$$F^1 \text{ offspring} \left\{ \frac{\text{Parent } \sigma \ 3L \times \text{Parent } \varphi \ 60S}{\varphi \ 127S \quad \varphi \ 222S \quad \varphi \ 254S} = 3S : 0L \right.$$

$$\text{Back cross:} \\ \text{Simplex} \times \text{recessive} \left\{ \frac{\sigma \ 3L \times \varphi \ 127S}{\sigma \ 255L \quad \varphi \ 256S \quad \varphi \ 313S \quad \varphi \ 314S} \quad \frac{\sigma \ 361L \times \varphi \ 127S}{\varphi \ 459L \quad \varphi \ 460L} = 3S : 3L \right.$$

$$\text{Cross:} \\ \text{Simplex} \times \text{extracted recessive} \left\{ \frac{\sigma \ 255L \times \varphi \ 127S}{\sigma \ 422S \quad \sigma \ 423L} = 1S : 1L \right.$$

$$\text{Cross:} \\ \text{Simplex} \times \text{simplex} \left\{ \frac{\sigma \ 422S \times \varphi \ 256S}{\varphi \ 573S \quad \varphi \ 461S \quad \varphi \ 462L} \quad \frac{\sigma \ 422S \times \varphi \ 127S}{\varphi \ 572S} = 3S : 1L \right.$$

While the experiment has been discontinued at this Station, a few more data have become available this spring, as No. 127 was again bred to a pure long-eared ram, though with the primary purpose of studying her performance as a twin bearer. She again dropped twins, a long-eared and a short-eared individual, which further establishes her simplex character with regard to the short-ear trait. No. 462 dropped her first lamb this year. As she is a pure recessive and bred to long-eared sire, a long-eared offspring was the result, as expected.

No. 422 and 572 were sent to Dr. C. B. Davenport,<sup>1</sup> of the Station for Experimental Evolution, of the Carnegie Institute, who bred the two and also bred the male to 12 long-eared females. Dr. Davenport reports that the 12 ewes bred to No. 422 all lambed, 10 of them having dropped twins and 2 of them triplets, the short-ear trait appearing in about one-half of the offspring, which supports previous data indicating his simplex character with regard to short ears.

<sup>1</sup> Acknowledgments are due to Dr. Davenport for valuable advice given during the prosecution of this work.





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# JOURNAL OF AGRICULTURAL RESEARCH

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## LIFE-HISTORY STUDIES OF CIRPHIS UNIPUNCTA, THE TRUE ARMY WORM

By JOHN J. DAVIS and A. F. SATTERTHWAIT,

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Bureau of Entomology*

### INTRODUCTION

For an insect so commonly injurious and widely known as the army worm (*Heliophila*) *Cirphis unipuncta* Haworth, comparatively little concerning its life economy is recorded, and the detailed experiments herein reported represent phases of the more exact studies begun by this branch of the Bureau of Entomology.

Late in July, 1914, the army worm appeared in very destructive numbers in Huron and Sanilac Counties, Mich., and from the progeny of larvæ collected in that locality eggs and larvæ were obtained with which a series of molting and feeding experiments was started at La Fayette, Ind. The feeding experiments, where exact records of the amount of corn foliage eaten in each instar were kept, are especially interesting and instructive, for it will be noticed that more than 80 per cent of all the foliage eaten during the entire life of the larva was consumed during the last larval instar, which corroborates previous field observations to the effect that army worms rarely become evident and destructive until they are nearly full grown.

### GENERATIONS OF THE ARMY WORM

During the past year (1915) *Cirphis unipuncta* was bred continuously throughout the season from moths collected from May 13 to 15, to determine the average number of generations annually. Moths of *C. unipuncta* were first observed at La Fayette the night of May 13 feeding on the honeydew produced by *Pulvinaria vitis* L.,<sup>1</sup> *Lecanium quercifex* Fitch,<sup>1</sup> and *Callipterus discolor* Monl. on white oak, and it is quite likely that these moths were the adults of larvæ overwintering in this latitude. Moths were placed in large breeding cages under natural outdoor conditions, and eggs were laid and the larvæ first observed on June 7.

<sup>1</sup> Kindly determined for the Bureau of Entomology by Prof. J. G. Sanders.

Pupæ were found in the cage on June 27 and the adult moths began to issue on July 8. The generation series was continued in another cage in which moths issued from July 8 to 10, eggs were found on July 14, larvæ as early as July 20, and the first adults on August 30. These adults, issuing between August 30 and September 8, were similarly confined, and eggs were first noticed on September 25 and larvæ on September 28. During the winter of 1915-16 they survived as partially grown larvæ and completed their growth in April, 1916. Thus, it is observed that in the latitude of La Fayette three complete generations may occur annually, and from numerous observations of 1914 and 1915 it is evident that in some seasons a partial fourth generation may be present. Likewise, the overlapping of generations may account for a partial fourth, although a complete fourth generation is seldom, if ever, produced in this latitude.

#### MOLTING AND FEEDING HABITS

The records of molts and of foliage eaten were made with larvæ confined in individual cages, three types of cages being used, namely, tin boxes, glass test tubes, and lantern globes (Pl. CVII, A). The tin boxes used for individuals 1 to 48 and 77 to 132 were of the common salve-box type, a 1-ounce size being used for the earlier stages and a 3-ounce size after the larvæ were about half grown. Individuals 49 to 76, inclusive, were reared in ordinary lantern-globe cages, with cheesecloth tops, placed on paper-padded saucers and containing corn foliage in bottles of water. Individuals 133 to 153 were reared in cotton-stoppered test tubes, which measured approximately 6 inches in length and 1 inch in diameter, smaller vials having been used for the first few instars. A single larva recently hatched was placed in each cage and fresh corn foliage given it as necessary. Frequent examinations were made to obtain as nearly as possible the exact hour of molting. Foliage uneaten was pressed, and from this the total amount eaten was computed for each larval instar, using for this purpose ordinary plotting paper squared to hundredths of an inch, by means of which a fairly accurate record of foliage eaten to thousandths of a square inch was obtained.<sup>1</sup>

The tin-box cages, 1 to 48 and 77 to 103, were kept indoors, and the lantern-globe cages, 49 to 76, and vial cages, 133 to 153, were kept on a latticed porch and were therefore under more nearly normal outdoor conditions. As the experiments were conducted for the most part during September and October, the nights were much cooler for the individuals on the porch, resulting in a noticeably longer life-cycle period for these than for the individuals kept indoors, where the coolness of the nights was much less evident.

<sup>1</sup> The authors take this occasion to acknowledge their indebtedness to Mr. D. G. Tower, of the Office of Cereal and Forage Insect Investigations, who assisted in measuring the pressed foliage and in making counts of eggs in the bodies of the moths.

For a summary of the data relative to the length of the various stages of *Cirphis unipuncta* and for the amount of foliage eaten in each instar, the reader is referred to Table I.<sup>1</sup> It will be noticed that the average lengths of the different stages for individuals in the lantern-globe cages and in glass vials were noticeably longer than for individuals reared in the tin boxes. This was not due to the difference in the style of cage, but can be accounted for, as mentioned above, by the fact that the lantern-globe and glass-vial cages were kept on a latticed porch, where the night temperature was much lower than in the laboratory where the tin boxes were kept. It will be noticed that the length of the egg stage is uniform in all cases, as they were kept under like conditions, and the average for 153 individuals was between approximately  $5\frac{1}{2}$  and  $6\frac{1}{4}$  days. As will be noticed, the length of the first five larval instars did not vary greatly one

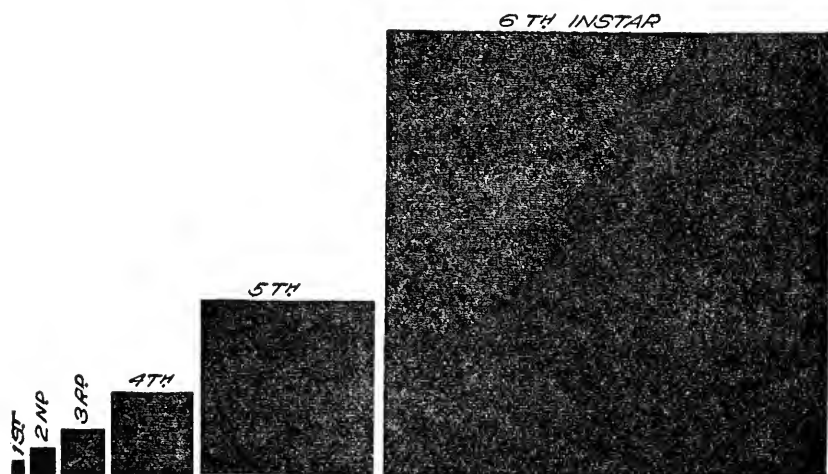


FIG. 1.—Diagram of relative amounts of foliage eaten in each larval instar by *Cirphis unipuncta*.

from the other, although the amount of foliage eaten in each of these instars was a gradual increase from nearly 0.03 of a square inch for the first instar to over 5 square inches for the fifth instar. The period for the sixth larval instar was noticeably longer than in any of the previous instars, being approximately two and one-half times as long; and the amount of foliage eaten in this instar was nearly seven times as much as in the fifth instar, and more than 80 per cent of all of the foliage eaten during the entire larval period. In other words, the total amount of foliage eaten by the larva during its entire life, an average from 108 individuals, was 41.394 square inches, and the average for the same individuals for the sixth instar alone was 34.128 square inches (see Table I and fig. 1). The remarkable voracity of the army worm during its last larval instar explains its sudden appearance in such enormous and destructive numbers only when it is nearly full grown.

<sup>1</sup> Owing to the size of the tables giving complete data for each individual, it is impossible to include them with this paper, but they are on file and may be obtained for reference by anyone interested.

TABLE I.—Comparison of the molting and feeding of *Cirphis unipuncta* at La Fayette, Ind., from August to November, 1914

Cage No. and date eggs were laid	Egg stage.		First instar.				Second instar.				Third instar.				Fourth instar.										
	Num-ber of indi-vidu-als.	Duration.	Num-ber of indi-vidu-als.	Duration.	Corn foliage eaten.	Num-ber of indi-vidu-als.	Duration.	Num-ber of indi-vidu-als.	Aver- age area.	Num-ber of indi-vidu-als.	Duration.	Num-ber of indi-vidu-als.	Aver- age area.	Num-ber of indi-vidu-als.	Duration.	Num-ber of indi-vidu-als.	Aver- age area.	Num-ber of indi-vidu-als.	Duration.	Num-ber of indi-vidu-als.	Aver- age area.				
1 to 48 (tin boxes). Aug. 22, 9.30 p. m.	48	Hours. 136.5 to 157.5	40	Hours. 78.787 to 98.891	Sq. in.	36	Hours. 53.219 to 65.254			Sq. in.	36	Hours. 56.246 to 74.115			35	Hours. 78.883 to 89.883			32	Hours. 68.880 to 75.938			31	Sq. in. 1.395	
49 to 76 (lantern globes): Aug. 26, 10 p. m., to Aug. 27, 10.30 a. m.	28	127.5 to 154.75	22	88.954 to 114.210	0.041	21	96.614 to 104.983	21	.121	.121	21	105.436 to 111.966	26	.346	26	68.880 to 75.938	21	1.159	21	68.880 to 75.938	21	1.159	21	1.159	
77 to 103 (tin boxes): Aug. 27, 9.45 p. m.	27	140.5 to 143.33	26	94.461 to 101.221	0.025	26	76.164 to 85.482	26	0.077	0.077	26	85.592 to 96.492	26	0.323	26	55.510 to 66.966	25	1.165	25	55.510 to 66.966	25	1.165	25	1.165	
104 to 132 (tin boxes): Aug. 28, 6 p. m., to Aug. 29, 6 a. m.	29	135.25 to 158.50	25	85.986 to 101.920	0.025	25	93.480 to 103.980	25	0.066	0.066	24	72.229 to 81.573	24	0.351	24	51.208 to 66.952	24	1.214	24	51.208 to 66.952	24	1.214	24	1.214	
133 to 153 (glass vials): Aug. 29, 9.30 p. m., to Aug. 30, 10.30 a. m.	21	158 to 131	19	136.284 to 142.250	0.031	16	94.500 to 100.578	16	0.134	0.134	15	51.733 to 62.666	15	0.339	14	58.393 to 70.285	14	1.101	14	58.393 to 70.285	14	1.101	14	1.101	
Mean	153	134.11 to 151.65	132	91.366 to 107.012	0.024	124	79.452 to 88.839	88	0.103	0.103	122	71.532 to 84.838	121	0.338	116	63.698 to 75.109	115	1.204	116	63.698 to 75.109	115	1.204	115	1.204	
Total	153		132			124		88			122		121		116			115		116		115		115	

TABLE I.—Comparison of the molting and feeding of *Cirphis unipuncta* at La Fayette, Ind., from August to November, 1914—Continued.

Cage No. and date eggs were laid.	Fifth instar.		Sixth instar.			Total corn foliage eaten.		Total length of larval stage.		Total length of pupal stage.		Total length of life cycle.	
	Num-ber of indi-vidu-als.	Aver- age area of corn foliage eaten.	Num-ber of indi-vidu-als.	Duration.	Corn foliage eaten.	Num-ber of indi-vidu-als.	Aver- age area.	Num-ber of indi-vidu-als.	Duration.	Num-ber of indi-vidu-als.	Duration.	Num-ber of indi-vidu-als.	Duration.
	Hours.	Sq. in.	Hours.	Sq. in.	Sq. in.	Sq. in.	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.	Hours.
1 to 48 (tin boxes): Aug. 22, 9.30 p. m. . . . .	29	107.784 to 116.280	24	134.104 to 144.066	29	36.262	24	537.6 to 591.6	24	367.780 to 393.780	29	1,683.320 to 1,698.393	
49 to 76 (lantern globes): Aug. 26, 10 p. m., to Aug. 27, 10.30 a. m. . . . .	21	96.583 to 106.428	19	391.287 to 311.274	20	28.26	20	786.864 to 893.4	19	702.864 to 716.304	19	1,632.144 to 1,656.318	
77 to 103 (tin boxes): Aug. 27, 9.45 p. m. . . . .	25	71.048 to 81.835	25	107.357 to 174.971	25	35.82	25	575.424 to 666.295	25	447.956 to 431.277	25	1,749.648 to 1,759.392	
104 to 132 (tin boxes): Aug. 28, 6 p. m., to Aug. 29, 6 a. m. . . . .	24	63.531 to 83.382	22	179.465 to 196.716	23	37.97	21	574.580 to 587.102	21	429.312 to 433.272	21	1,151.112 to 1,165.752	
133 to 153 (glass vials): Aug. 29, 9.30 p. m., to Aug. 30, 10.30 a. m. . . . .	13	76.725 to 86.515	13	297.675 to 391.618	13	40.982	13	736.764 to 742.902	13	761.928 to 768.768	13	1,612.816 to 1,616.818	
Mean . . . . .	112	81.756 to 91.822	103	293.346 to 212.261	110	34.128	108	626.52 to 641.333	102	512.328 to 518.976	107	1,276.488 to 1,287.432	
Total . . . . .													

a This record does not include foliage eaten in first two instars.

It will be noticed that the egg stage approximated 6 days, the larval stage required about 26 days, and the pupal stage 21 days for over 100 individuals and that the average length of life cycle for these was 53 days. It will also be noticed that for the cages kept indoors, which would approximate late spring, summer, or early fall conditions, the total length of the larval period averaged about 23 days; that of the pupal period about 17 days, and that of the entire life cycle approximately 47 days; while for the individuals kept on the back porch, which approximated early spring or late fall conditions, the average length of the larval period was about 31 days, the pupal period 30 days, and the total life cycle approximately 68 days. The larva has six instars, molting five times previous to pupation. However, this may vary, for among the 107 individuals reared through to the adult stage one had seven instars, molting six times.

In the outbreak in Michigan, which claimed attention in the latter part of July, 1914, oats, barley, corn, grass, alfalfa, and beets were attacked in the order mentioned. Oats and barley were backward on account of an unusual June freeze and for this reason were more succulent and attractive to the army worms. In the case of small grains, especially oats, the relative amount of injury was much greater in proportion to the amount of actual food eaten than for such crops as corn, for in the former case the grain was clipped from the stalks by the worms, leaving most of the grain uneaten and the ground whitened with the grain heads.

At the time of the outbreak mentioned above the corn was 2 feet in height. Montgomery<sup>1</sup> has shown that mature corn plants have a foliage area of 927.8 to 1,912.9 square inches, with an average of 1,200 square inches. Corn plants 2 feet in height would have at the most not more than one-twelfth the foliage area of a mature plant; hence, it can be said with comparative assurance that a corn plant such as was found in Michigan during the 1914 outbreak would have not more than 100 square inches of foliage. Since one larva would eat 41.4 square inches, it would require five larvæ to devour two corn plants. With 8,890 corn plants to an acre ( $2\frac{1}{2}$  plants to a hill and  $3\frac{1}{2}$  feet each way), it would require 21,473 worms to destroy an acre of corn 2 feet in height. Although seemingly a large number of worms, this number represents only the progeny of probably not more than 40 female moths.

According to the observations of the writers, the eggs laid at night in clusters of 25 to 134 on grass or other host plant between overlapping leaves fastened together or between the leaf sheaths, often none of the eggs or only a small part of the mass being visible (Pl. CVII, B). They are fixed to the leaf by means of a glutinous secretion which when dry is white and flaky. The largest number of eggs laid by a single female was 254 (see Table II), and in all cases where

<sup>1</sup> Montgomery, E. G. Correlation studies of corn. *In* Nebr. Agr. Exp. Sta. 24th Ann. Rpt., 1910, p. 108-159, illus. 1911.



the body of the dead female was examined many more eggs in all stages of development were found in the ovaries. In some cases more than 800 developed and undeveloped eggs were contained in the body of a single female.

The newly hatched larvæ first eat the eggshells, but apparently do not eat the white substance by which the eggs were attached. Later they feed on the tissues of the corn leaf on which they are resting, destroy the parenchyma, and leave the other leaf surface as a transparent membrane (Pl. CVII, C). Later, they feed from the edge of the leaf, devouring all the leaf tissue.

In the first instar the larvæ, if slightly disturbed, give themselves a compound twist, "humping" the body and drawing the head and thoracic segments around, bringing the ventral surface forward, and clinging by the pseudolegs and anal claspers. If further disturbed, they drop on a silken thread, and in this twisted position resemble balls of frass, losing all semblance of the larval form. Sometimes the larvæ contort themselves with a snap or fling, without the silken attachment, which fact seemed to explain the loss of an occasional individual in the experiments.

TABLE II.—Eggs laid by individual females of *Cirphis unipuncta* at La Fayette, Ind., August to September, 1914

Cage No.	Aug. 26.	Aug. 27.	Aug. 28.	Aug. 29.	Aug. 30.	Aug. 31.	Sept. 1.	Sept. 2.	Sept. 3.	Sept. 4.	Sept. 5.	Sept. 9.	Total.	Eggs in body of dead female.	Total eggs laid and in body.
K <sup>1</sup> .....				77	12	( <sup>2</sup> )							89	(1)	.....
L <sup>1</sup> .....		28	5	90	20		4	71	36				254	91	345
L <sup>2</sup> .....				25	41	92	5	48	3	( <sup>2</sup> )	( <sup>2</sup> )		214	55	269
L <sup>3</sup> .....					134	39	48	( <sup>2</sup> )					221	(1)	.....
M <sup>1</sup> .....	18	25	{ 15 34 63	43	21	( <sup>2</sup> )							219	62	281
M <sup>2</sup> .....	15					( <sup>2</sup> )								15	431
M <sup>3</sup> .....				10			54					( <sup>2</sup> )	64	(1)	.....

<sup>1</sup> Abdomen of dead moth not examined.

<sup>2</sup> Died.

The use of a silk thread by larvæ when disturbed occurred with less frequency in the second instar. After dropping, the first-instar larvæ remain inactive for a moment before attempting to crawl away; and in later instars they swing the head or fore part of the body vigorously to one side and feign death. During the fifth molt, a disturbed larva feigned death for five minutes.

During the first and second instars the larvæ walk in a looping manner, but this characteristic is lost in the third and succeeding instars.

As the larva approaches a molt, the condition is recognizable by the largeness of the body diameter as contrasted with that of the head. When within a few hours of the molt, the larva habitually anchors its anal

claspers to whatever it is resting upon, usually a rigid object rather than the foliage. In some cases—for more than 14 hours in the third instar, 21 hours in the fourth instar, and 56 hours in the fifth instar—before the molt occurs, the apparent movement of the ocelli may be observed from their normal position to a position entirely behind the mask and within the stretched integument of the first thoracic segment. Before the rupture of the integument takes place, the old mask is in the position of a muzzle in relation to the withdrawn head. The mask separates from the body integument, which splits along the median dorsal line for perhaps four segments. The larva moves its head vigorously from side to side and brushes the mask off against some object or its own body. The withdrawal of the body from the integument begins with the muscular action of the body, the larva ultimately crawling forward a distance about one-fourth or one-half its length, and after resting thus for a time if undisturbed, it will almost always turn around and eat its newly molted skin. In no instance was a mask observed to be eaten. Immediately after molting, the head and anal segments are white, the body and cast skin moist, and the head noticeably larger in diameter than the body.

One larva was observed to eat its cast skin in four to four and one-fourth minutes. Another, which required only three minutes, held the cast skin with the front pair of legs, remaining stationary and pulling the skin to it, using the second pair of legs to hold to the foliage for the first minute and utilizing them to help manipulate the partly eaten skin. Another, in the third instar, took eight and one-half minutes to eat all but a trace of its freshly cast skin. It used its front legs almost continuously and the second pair of legs about half the time in holding the skin while eating it. Another had occupied about five minutes in this process when it was accidentally interrupted.

When the mature larva has finished feeding, the alimentary tract is soon emptied and shortens up to a marked degree, the larva then preparing to spin a thin cocoon. In nature the larva burrows into the ground or among or under trash. In the cages soil was supplied to some; others had only the paper on which they were lying, others were among corn foliage, and some had nothing whatever to utilize for a cell. In those instances where soil was supplied, the larva spun an appreciable quantity of silk as lining for its cell. Where paper was cut, the effort was appreciable, but not enough silk was used to form more than a shallow cup. Where there was foliage, it was chewed up and mixed with silk, but with scant resemblance to a cocoon; where no material was furnished, the silk was not evident, although pupation and the issuance of adults seemed to be equally normal.

Just before pupation a deep pit develops in the emargination of the posterior dorsal line of the mask, and transverse ridges on the dorsal portion of abdominal segments 5, 6, and 7 show distinctly through the larval skin. These ridges, interrupted at the ends, are marked with 20

to 22 blunt teeth, which appear as transverse striæ. Also the prothoracic spiracles of the pupa are observable just dorsad of those on the larva through the mesothoracic integument of the larva as red-brown chitinized spots. Two or three minutes before the skin begins to split, the dorsum of the second segment of the thorax had changed its shape conspicuously, suggesting a scutum or scutellum extension. The skin on the fifth and following abdominal segments appears to be shriveled up and ready to drop off about 15 minutes before pupation. A red mark observed on the clypeus of the new pupa is not observable through the larval mask.

Pupation, so far as the molt of the last larval skin is concerned, was observed to take place in about a minute. The integument splits along the median dorsal line of the thorax and the pupa vigorously works itself out. The mask splits along the inner seams of the genæ, leaving a triangular piece above the clypeus, and the whole remains clinging to the cast skin after the pupa has escaped. The lining of the esophagus was cast with the mask. The compound eyes continued as dark spots on the new pupa for several minutes after the mask had split. The wing pads at time of escape of pupa from exuvium—about four minutes after the mask first split—reached 8.25 mm. back from the apex of the head. The red-brown spot on the clypeus of the pupa is slightly above the point from which the esophagus was cast. There was no apparent function for this spot. The prothoracic spiracles of the pupa are red-brown, as evidenced through the larval skin a few minutes before pupation, and those posterior lack the red color but have slightly dusky rims. About 14 minutes after the molting the wing pads reached 9.60 mm. back from the apex of the head. From other observations the color of the prothoracic spiracles of the pupa may vary to crimson and other spiracles to pinkish red or rosy with a fine line of dark red at the rim. The pupa is at first cream-colored; in about 18 minutes after issuing it is a pale salmon, and in 25 minutes it begins to get brown. After that it browns up rapidly, but one and one-fourth hours afterwards it has not become full mahogany brown. The anal spines appear to be very useful in "kicking off" the exuvium. About 15 minutes after pupation the fat body shows through the wing pads and is irregularly assembled at ends of abdominal segments. About 30 minutes after pupation the two abdominal segments next behind the wing pads are getting rosy bands, and the last three segments are almost solidly the same color. The dorsum of the abdomen is of a dark-rose color, with two or three segments on the dorsal aspect having brown, roughened, chitinized edges. About 45 minutes after molting, the abdomen behind the wing pads is nearly uniformly rosy. The fat body now appears in rings through the wing pads. About one and one-fourth hours after pupation, the rose color gradually changes through dark salmon to light red-brown. The abdomen continues light red-brown for two and one-fourth hours after, but no red has begun to appear on the wing pads. Within three and

three-fourths hours the abdominal segments are nearly the normal shining red-brown.

Immediately after pupation the pupa stretches itself longitudinally almost to bursting, remains so from 12 to 15 minutes, and has been observed to contract and then extend itself again before resuming a natural appearance. On three pupæ immediately after pupation there was a transverse red-brown mark on the clypeus near its base and between the compound eyes. Each of these pupæ, while nearly white, showed a red-brown spot dorsad of the antennæ, which is the prothoracic spiracular spot.

The moth, immediately after emerging from the pupal shell, often carries a drop of clear fluid at its mouth. It then runs for a short distance. The wings require about 20 minutes to become fully expanded, with the upper surfaces folded together and hanging, and within one and a quarter hours they are in natural position flat on the back or slightly tectiform.

#### DESCRIPTION OF STAGES<sup>1</sup>

##### THE EGG

When first laid the eggs are perfectly smooth, shining milky white, and without any trace of sculpturing. Later the color changes through cream to flesh color, and just before hatching to a leaden cast. From a dorsal view they are apparently symmetrically spherical, but when laid in rows or masses, as is usually the case, they become compressed on two sides. Length, dorsal view, 0.542 to 0.561 mm.; width, dorsal view, 0.425 to 0.464 mm.

##### THE LARVA

**FIRST INSTAR.**—Head pale vandyke brown, shining, its posterior margin deeply emarginate. Ocelli blackish, 12 in number, 6 on each side of the head, 5 of which are arranged in a semicircular form, the lowest of the 5, however, being separated by a space so that it appears paired with the sixth, which is located near the base of antenna. Cervical shield on prothoracic segment pale dusky. When first hatched the body is whitish, later becoming tinged with green, due to chlorophyll from the foliage eaten. Entire body sparsely clothed with moderately long fine hairs, which are about as long as half the width of the body, those projecting from the head and sides of the body whitish, the dorsal hairs blackish, and the body hairs on more or less conspicuous black tubercles. The fore pair of abdominal legs are somewhat atrophied, which may account for the characteristic looping walk of the first and second instar larvæ.

Just before the first molt the head is very dark brown to almost black, and the transverse cervical shield is dusky brown. There is a clear white, shining area with several blackish dots in a row on each side extending from the anterior end of the segment to a distance about equal to the width of the cervical shield, anterior to the latter, which represents the head and ocelli of the second-instar larvæ. The ground color of the body is of a decided greenish tint, paler toward the posterior end. The body segments bear seven alternate brown-ocher and white longitudinal stripes on each side of the whitish median dorsal line; the ventral surface is white. The dorsal lines are more or less obliterated or inconspicuous on the thoracic segments. Hairs much less conspicuous than early in the instar. The thoracic legs black, the pseudo and anal legs pale dusky.

Measurements, average of two individuals, as follows: Recently hatched larva, length of body 2.11 mm., width of first thoracic segment 0.35 mm., of cervical shield

<sup>1</sup>All descriptions and measurements, unless otherwise noted, are from living specimens.

0.25 mm., of abdominal segments 0.28 mm., width of head 0.35 mm. Just before first molt, length of body 4.02 mm., width of prothoracic segment 0.464 mm., width of abdominal segment 0.474 mm., length of cervical shield 0.310 mm., width 0.116 mm., width of head 0.35 mm., length of cephalic hairs 0.155 mm., abdominal hairs 0.080 mm.

SECOND INSTAR (immediately after first molt).—Head very pale, shining translucent, and with a very faint tint of raw sienna; no apparent reticulate markings such as appear in later instars; tips of mouth parts brownish to black. Ocelli black and arranged as in the first instar, but more prominent and not so closely placed. Cervical shield shining, translucent, and inconspicuous, and thoracic legs faintly dusky translucent. Ground color of anterior half of body pale green, of the posterior half whitish and with stripes as in preceding instar, but the darker lines more of a yellow-ocher color. Ventral surface whitish or greenish white. Pseudo and anal legs pale translucent. The fine hairs covering the body are whitish and placed on rather conspicuous black tubercles.

Just before the second molt, the head a shining, light raw umber; mouth parts darker. The ground color of the prothoracic segment whitish, due to the head of the third-instar larva, which is plainly visible through the translucent skin. Body gradually narrowing to anal segment. Otherwise similar in markings to the recently molted second-instar larva, except that the legs are slightly darker, and the lowest brown longitudinal line is paler, tending to yellowish orange.

Measurements, average of two individuals, as follows: Recently molted larva, length of body, 3.10 mm., width of prothoracic segment 0.531 mm., width of proanal segment 0.368 mm., width of head 0.58 mm., length of longest cephalic hairs 0.348 mm.

SECOND INSTAR (just before second molt).—Length of body 6.15 mm., width of prothoracic segment 0.71 mm., abdominal segments vary in width from 0.928 mm. for the first abdominal segment to 0.74 mm. for the proanal segment; width of head 0.56 mm.

THIRD INSTAR (just before third molt).—The head shows the reticulation or brownish mottlings illustrated by Forbes.<sup>1</sup> Prothoracic segment transparent, the pale reddish brown reticulations and ocelli on the head of the fourth-instar larva plainly visible beneath. Body markings as in preceding description, excepting that the second dorsal brown band has broken into two brown bands and one whitish band; the third brown band has a paler more or less conspicuous line along its median; the brown band at base of legs almost obliterated; the longitudinal stripes become very faint at the anterior and less so at posterior end. The general color of the body is greenish at anterior end and cream tinted posteriorly. Ventral surface greenish white. Body hairs whitish, except those on dorsum, which are blackish. First pair of abdominal legs now fully developed. Otherwise as in previous instars.

Measurements, average of two specimens, as follows: Just before third molt, length of body 10.35 mm., width of prothoracic segment 1.26 mm., of anal segment 1.05 mm., of head 0.95 mm.

FOURTH INSTAR (just after third molt).—Head pale umber with the reticulated markings of raw sienna, as in third instar, but more prominent. General color of body Nile green, paler posteriorly. The longitudinal stripes as in preceding instar, except that lines below the line of spiracles have become entirely obliterated, the color below the spiracles being whitish green to Nile green. The dorsal lines are inconspicuous or indistinct at their extremities. Otherwise as in preceding instars.

FOURTH INSTAR (just before the fourth molt).—Head pale translucent with reticulated areas of dusky brown, darker than earlier in instar. The prothoracic segment swollen, showing head of larva of fifth instar; a light raw sienna, the reticulated mark-

<sup>1</sup> Forbes, S. A. A monograph of insect injuries to Indian corn. Part II. 23d Rpt. State Ent. Ill., p. 84, fig. 63 b. 1905.

ings on it plainly visible. The longitudinal stripes arranged as in preceding description, the color of the lines becoming intermixed and less distinct; the white spiracular stripe just below the line of spiracles with an interrupted ochereous line, not observed in preceding instar. The general body color is pale greenish on thoracic segments, the remaining segments gradually changing to pale yellowish brown. Spiracles black, surrounded by rather conspicuous whitish areas, those on prothoracic and proanal segments largest.

Measurements, average of two individuals, just after third molt: Length of body 10.7 mm., width of prothoracic segment 1.47 mm., of anal segment 1.28 mm., of head 1.45 mm. Just before fourth molt: Length of body 15.0 mm., width of prothorax 1.86 mm., of anal segment 1.55 mm., of head 1.47 mm.

FIFTH INSTAR (just after fourth molt).—Head as described for previous instar. Ocelli become more distant with growth of head, the posterior one of the two near base of antenna reduced to an inconspicuous spot, much resembling a seta spot. The longitudinal stripes on abdomen as in preceding instar, except that just below the line of spiracles is a narrow white line followed by another rather narrow line of reddish burnt umber, the underside of body cream-colored or with faint greenish tint; dorsal surface appearing mottled with brownish markings on pale or cream-colored background. The median slit of spiracle is more conspicuous and shows as a paler whitish area. Otherwise as in preceding instar.

FIFTH INSTAR (nearly full-grown fifth-instar larva while feeding).—The entire body from dorsal view a dirty greenish color with pink tint, the former darkest near central portion of body and along median dorsal lines and the latter more prominent at the extremities, obliterated by the dull-green coloration of the dorsum. Below the line of spiracles is a pale pinkish green longitudinal line bordered on either side by a narrow whitish line, above and below which the more or less mottled dusky-green color predominates. Spiracles as before, but of a more velvety black. Legs as before, but the pseudolegs with dusky encircling bands at extreme base visible when legs are fully extended. Otherwise as previously described.

FIFTH INSTAR (just before fifth molt).—Head and prothoracic segments of same general appearance as in preceding instar just before molting. From a dorsal view the general color is pale yellowish green to cream, the longitudinal lines being very faint with no definite outline. The broad longitudinal dark stripe just above line of spiracles contrasts strongly with the whitish or cream-colored area below the spiracles. Other markings as in preceding instar.

Measurements, average of two individuals, just after fourth molt: Length of body 13.85 mm., width of prothoracic segment 2.13 mm., of anal segment 1.64 mm., of head 2.30 mm. Nearly full grown and while feeding: Length of body 20.7 mm., width of prothoracic segment 2.59 mm., of head 2.44 mm. Just before fifth molt: Length of body 20.55 mm., width of prothoracic segment 2.90 mm., of proanal segment 2.32 mm., of head 2.38 mm.

SIXTH INSTAR (about full grown, Pl. CVII, *D*, but still feeding).—Head reticulated and mottled as before, but median suture with border of dark raw umber. Cervical shield more prominent and shining, covering almost the entire dorsum of the prothoracic segment. General color from dorsal view dirty-pale brown, paler at posterior third. The general color varies in different individuals, some being very pale while others appear very dark and in some cases even almost black. At the beginning of this instar the general color was pale with a distinct pinkish tint and the median-dorsal area was dull green, the pinkish shades predominating at the posterior extremity. A median dorsal and two conspicuous white lines laterad on dorsum of prothorax, each of the latter bordered on its inner side by a narrow dark-brown area. These white lines are extensions of longitudinal lines extending the length of the body, but are much more prominent and distinct on the prothoracic segment. The median dorsal line or stripe dark brown, its median interrupted at

intervals by narrow white dashes, these the remnants of the white median line of previous instars; laterad to this median stripe is a pale line which becomes interfused with the former along its border; below is an interrupted dark-brown stripe followed by a white stripe, and this by a rather conspicuous dark-brown stripe of the same width as last and just above line of spiracles. Below the line of spiracles is a conspicuous yellowish or cream-colored stripe with a more or less pinkish tint, contrasting with the dark-brown line above and the dusky-brown area beneath. Ventral surface of body uniformly pale, but slightly brownish or dusky, the dorsum appearing mottled and the pinkish shades rather conspicuous. Body sparsely covered with very fine hairs placed on minute black tubercles. Spiracles as in preceding instar. Legs pale dusky at joints and at tips. Pseudolegs pale and when extended a conspicuous black band is visible on outer side and extending halfway round the legs at their base.

Measurements, average of two individuals, shortly after fifth molt: Length of body 24 mm., width of prothoracic segment 3.41 mm., of head 3.48 mm. Full grown: Length of body 35 mm., width of prothorax 3.8 mm., of widest abdominal segment 6.0 mm., of anal segment 3.5 mm., of head 3.4 mm.

Individual 75, which had an extra instar—that is, seven—agreed in general markings with the description for the fifth-instar larva given above. Measurements for this individual just before sixth molt as follows: Length of body 21.2 mm., width of prothoracic segment 2.9 mm., of widest abdominal segment 4.0 mm., of proanal segment 2.4 mm., of head 2.4 mm.

The head widths are apparently good and substantial characters for distinguishing larvæ of different instars, the width varying only slightly in different individuals and never varying in any instar for the same individual. In Table III are given the average head widths for each instar, the records here given showing only those measurements actually recorded in descriptive notes, although many other corroborative measurements were made at frequent intervals.

TABLE III.—*Width of head (in millimeters) of Cirphis unipuncta at different stages of growth*

Number of individual in series.	First instar.	Second instar.	Third instar.	Fourth instar.	Fifth instar.	Sixth instar.
11.....	0.348					
(?).....	.348					
45.....		0.581				
42.....		.581				
47.....		.581				
48.....		.542				
146.....		.581				
152.....		.581				
39.....			0.968			
44.....			.929			
9.....				1.432		
38.....				1.471		
22.....				1.471		
30.....					2.326	
36.....					2.284	
144.....					2.361	
153.....					2.400	
133.....					2.439	
61.....						3.484
123.....						3.400
Average....	.348	.574	.948	1.458	2.362	3.442

## THE PUPA

When fully colored of a shining mahogany brown, and comparatively smooth. In general appearance not unlike other noctuid pupæ. Dorsum of thorax with noticeable transverse wrinkles, the mesothorax with a more or less distinct smooth line along the median dorsum. Bases of first four abdominal segments on dorsum and of fifth, sixth, and seventh segments on venter rather indistinctly and sparsely punctured; the fifth, sixth, and seventh segments with a ridge near the anterior border on dorsum which reaches nearly to spiracles on each side, the posterior margin of this

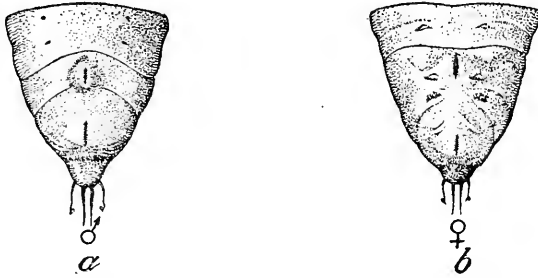


FIG. 2.—Posterior extremity of male and female pupa: *a*, Male; *b*, female.

ridge being denticulate or deeply crenulate, the teeth directed caudad and most prominent at median dorsum and gradually becoming less distinct laterally. The male and female pupæ are readily separated by a comparison of the accompanying illustrations (fig. 2).

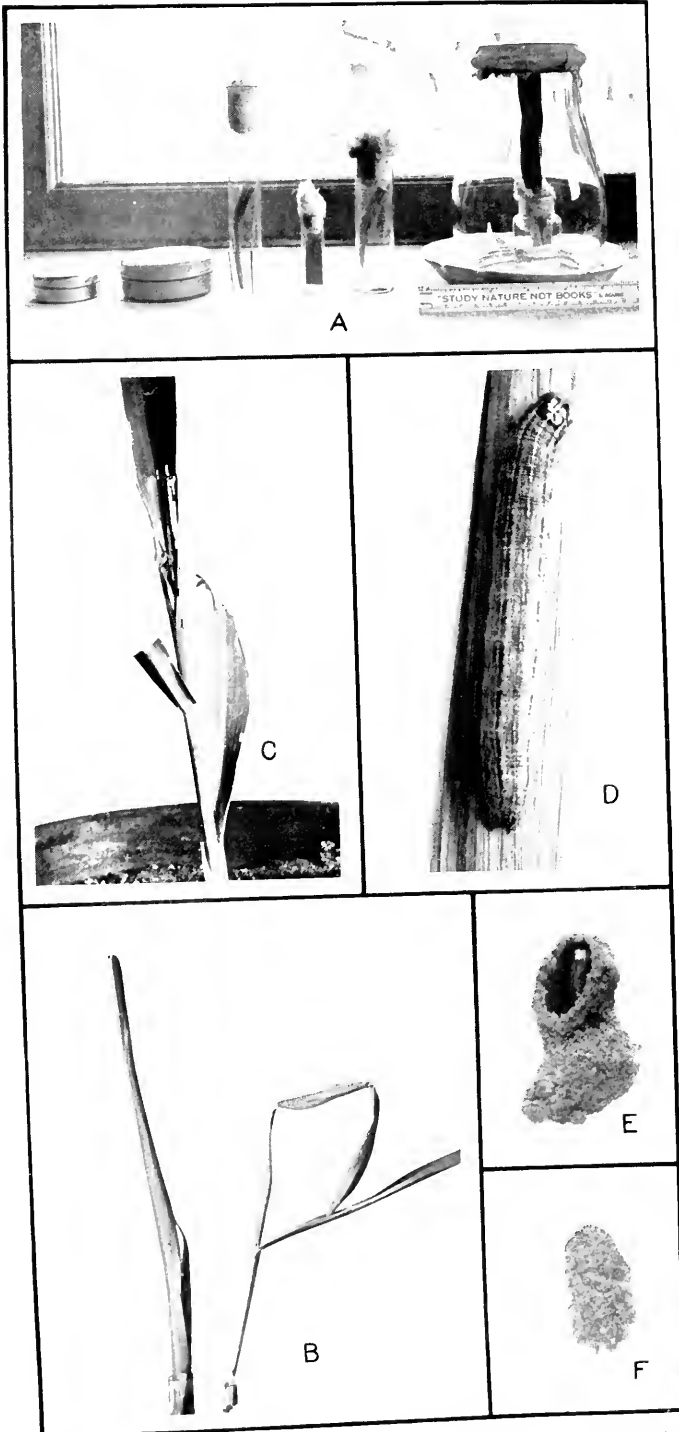
Measurements: Length 19.3 mm.; width at tip of wings of male 6.2 mm., of female 7.0 mm. Length of the two prominent robust spines at tip of body in male 0.55 mm., in female 0.7 mm.; a more slender and more or less hooklike spine on each side of the larger one is shorter.





PLATE CVII

*A*, Cages for rearing *Cirphis unipuncta*; *B*, leaves glued together after the eggs have been deposited; *C*, characteristic leaves partly eaten by first-instar larvæ; *D*, full-grown larva; *E* and *F*, characteristic pupal cells.





## INFECTION OF TIMOTHY BY PUCCINIA GRAMINIS

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It has been shown a number of times that *Puccinia phleipratensis* Eriks. and Henn. can infect oats (*Avena sativa*) and rye (*Secale cereale*), and it has also been shown recently that it can infect barley (*Hordeum vulgare*; (8, p. 213).<sup>2</sup> Inoculation of timothy (*Phleum pratense*) with *Puccinia graminis* was reported by Eriksson (2, p. 71), Johnson (3, p. 9), Mercer (6, p. 22), Stakman and Jensen (8, p. 213), and others as giving only negative results. Carleton, however (1, p. 62), succeeded in infecting *Phleum asperum* with *P. graminis avenae*.

The timothy-rust problem offers a good field for investigating the possible origin and developmental tendencies of biologic forms. The rust can infect oats, rye, barley, and a number of wild grasses; but morphologically it differs from *P. graminis*, and its ability to infect barberry (*Berberis vulgaris*) regularly is still a matter of doubt (3, p. 11). From its close similarity to *P. graminis avenae*, however, it seems reasonable to suppose that it may possibly have developed from some form of *P. graminis*. Since *P. phleipratensis* resembles *P. graminis avenae* parasitically more closely than any other biologic form of *P. graminis*, it would seem that infection of timothy with *P. graminis avenae* might be possible. For this reason the writers made a very large number of inoculations on a number of strains of timothy.

All inoculations were made on seedlings from 3 weeks to 3 months old. The leaves were first thoroughly moistened and then inoculated heavily with urediniospores of *P. graminis avenae* originally isolated from *Dactylis glomerata* and then kept on oats in the greenhouse for 14 months, having been transferred 30 times during that period. The rust had been used extensively in a large number of inoculation experiments, and the fact that it was a normal strain of *P. graminis avenae* had been well established. After inoculation the pots containing the seedlings were put in pans containing a small amount of water and were then kept covered with bell jars for 48 hours. At the end of that time they were removed and kept on an ordinary greenhouse bench. Inoculations were made with other biologic forms of *P. graminis* also; none of these, however, resulted in infection, therefore serving as checks. The "ordinary" timothy seed used was obtained from the Minnesota

<sup>1</sup>In cooperation with the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

<sup>2</sup>Reference is made by number to "Literature cited," p. 816.

Seed Laboratory, and was selected from seed trade samples. The Cornell and Minnesota selections or strains were obtained from the Section of Plant Breeding, Division of Agronomy and Farm Management, Minnesota Experiment Station. A summary of the results of inoculations is given in Table I.

TABLE I.—Results of inoculations with *Puccinia graminis* on *Phleum pratense*

Date of inoculation.	Source of urediniospores.	Strain of <i>Phleum pratense</i> inoculated.	Number of leaves inoculated.	Number of leaves infected.	Date of inoculation.	Source of urediniospores.	Strain of <i>Phleum pratense</i> inoculated.	Number of leaves inoculated.	Number of leaves infected.
1915. Dec. 8	<i>P. graminis avenae</i> from <i>Avena sativa</i> .	Ordinary timothy.	48	0	1916. Mar. 23	<i>P. graminis avenae</i> from <i>Avena sativa</i> .	Minnesota 79	170	8
1916. Jan. 25	.....do.....	.....do.....	50	5	Mar. 3	<i>P. graminis tritici</i> from <i>Hordeum vulgare</i> .	Ordinary timothy.	28	0
Feb. 7	.....do.....	.....do.....	76	2	Mar. 16	.....do.....	.....do.....	360	0
Feb. 18	.....do.....	.....do.....	234	3	Mar. 30	.....do.....	.....do.....	100	0
Mar. 2	.....do.....	Cornell 1671.	248	7	Mar. 4	.....do.....	.....do.....	30	0
Do. ....	.....do.....	Cornell 1743.	140	6	Do. ....	.....do.....	Cornell 1687.	88	0
Do. ....	.....do.....	Cornell 1611.	160	4	Do. ....	.....do.....	Minnesota 63	168	0
Do. ....	.....do.....	Cornell 1715.	120	0	Apr. 6	<i>P. graminis secalis</i> from <i>Secale cereale</i> .	Cornell 1715.	78	0
Do. ....	.....do.....	Cornell 1650.	96	0	Do. ....	.....do.....	Cornell 1635.	60	0
Do. ....	.....do.....	Cornell 1676.	134	1	Do. ....	.....do.....	Cornell 1630.	66	0
Do. ....	.....do.....	Minnesota 78	196	5	Do. ....	.....do.....	Cornell 1611.	60	0
Do. ....	.....do.....	Cornell 3230.	170	1	Mar. 17	<i>P. graminis secalis</i> from <i>Hordeum vulgare</i> .	Cornell 3230	72	0
Do. ....	.....do.....	Cornell 1687.	100	4	Mar. 8	<i>P. graminis secalis</i> from <i>Elymus virginicus</i> .	Cornell 1676	50	0
Do. ....	.....do.....	Cornell 1777.	94	0	Do. ....	.....do.....	Cornell 1687.	68	0
Do. ....	.....do.....	Cornell 1620.	240	1					
Do. ....	.....do.....	Minnesota 50	180	4					
Do. ....	.....do.....	Minnesota 70	150	0					
Do. ....	.....do.....	Minnesota 79	200	0					
Do. ....	.....do.....	Minnesota 63	186	6					
Do. ....	.....do.....	(G. Bros. 250r).							
Do. ....	.....do.....	Minnesota 63	140	0					
Do. ....	.....do.....	(G. Bros. 380r).							
Mar. 23	.....do.....	Minnesota 77	140	0					

It will thus be seen that successful infection resulted in 14 of the 22 trials with *P. graminis avenae* and it occurred on at least 11 different selected strains which when grown in the greenhouse varied considerably in type and vigor. Unpublished results obtained by Mr. M. N. Levine, a graduate student in the University of Minnesota, corroborate the work done by the writers. Mr. Levine's inoculations were made with another strain of *P. graminis avenae*, and, although there is probably little or no difference between one strain of this rust and another, it is interesting to know that the results obtained are not due to the peculiarities of the particular rust strain used. Inoculations on oats with the spores produced on timothy resulted in the formation of typical pustules in about eight days. None of the 774 timothy leaves inoculated with *P. graminis tritici* produced pustules, and none of the 454 inoculated with *P. graminis secalis* became infected, although the writers are not convinced that these transfers are impossible.

It is quite evident both from the percentage of successful infections and from the character of the infection that timothy can not be considered a congenial host for *P. graminis avenae*. The total number of leaves inoculated was 3,270 and only 57 became infected, only 1.47 per cent. The pustules were always small, ranging in size from mere dots to pustules 0.3 mm. in diameter. On the older leaves they were often surrounded by a small dead area, indicating a certain degree of hypersensitiveness, while on younger leaves they often appeared to develop quite normally except in size. Four or five pustules sometimes developed on the same leaf, giving the appearance of fairly successful infection. The incubation period varied from 8 to 12 days. The spores were considerably smaller in size than those of *P. graminis avenae*, but they were larger than those of *P. phleipratensis*. The spores of *P. graminis avenae* are also reduced in size on barley; the character of infection is somewhat the same as that on timothy and the spores become almost identical in size. Comparative measurements of spore lengths are given in Table II.

TABLE II.—Length of urediniospores of *P. graminis avenae* and *P. phleipratensis*

Rust organism.	Host on which measured.	Length limits.	Mode.
<i>P. graminis avenae</i> .....	<i>Avena sativa</i> .....	24 to 35.52.....	29.44
Do.....	<i>Phleum pratense</i> .....	20.16 to 32.64.....	25.60
Do.....	<i>Hordeum vulgare</i> .....	20.80 to 32.96.....	25.60
<i>P. phleipratensis</i> .....	<i>Phleum pratense</i> .....	16 to 28.80.....	21.76

Although the size of the spores is decreased on timothy, it becomes normal the first generation when the rust is transferred back to oats. The decrease in size is probably to be regarded only as a stunting due to unfavorable environment, since it has been previously shown that spores of *P. graminis* produced on an uncongenial host tend to become smaller than on a congenial host (7, p. 31). The color of the spores remains constant on oats and timothy and they can thus be distinguished very easily from spores of *P. phleipratensis*. Spores of *P. graminis avenae* are a bright cadmium-yellow in color, while those of *P. phleipratensis* are much duller, sometimes almost gray.

The fact that *P. graminis* can infect timothy raises the question as to whether *P. phleipratensis* may not have developed from some biologic form of this rust. Only speculation is possible at the present time, and a discussion of the possibilities is therefore probably useless. Nevertheless it is significant that *P. graminis avenae*, which now seems a possible source of the rust, produces urediniospores of very different shapes and sizes on the same plant and in the same pustules, thus conceivably indicating a tendency toward instability. The rust also has a wide range of hosts, in that while occurring commonly on one cereal, oats, and being

capable of infecting two others, barley and rye, it is also capable of infecting many wild grasses in this country and Europe. Until further, more extensive attempts are made to infect barberries with teliospores of *P. phleipratensis* and until the possibilities of developing experimentally a strain of *P. graminis* on timothy have been exhausted, work is more desirable than words, but the fact that *P. phleipratensis* can infect three of the cereals and a number of grasses and that timothy can be infected by *P. graminis avenae* may possibly indicate that timothy rust, as Kern (4, 5) has previously suggested, may not be so far removed from *P. graminis* as has sometimes been supposed.

## SUMMARY

(1) It has been possible by means of artificial inoculations to infect various strains of timothy with *Puccinia graminis avenae*.

(2) Timothy exerted an appreciable effect on the morphology of spores of *P. graminis avenae*, reducing them considerably in size. Practically identical results were obtained by transferring the rust to barley.

(3) The rust was subnormal in vigor on timothy, the pustules always remaining small.

(4) The facts recorded in this paper are suggestive of the possible origin of *P. phleipratensis*.

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# CONTROL OF THE POWDERY DRYROT OF WESTERN POTATOES CAUSED BY *FUSARIUM TRICHOETHECIOIDES*

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## INTRODUCTION

Wherever potatoes (*Solanum tuberosum*) are grown, storage-rots occur. These rots are in the majority of cases caused by wound parasites which attack the potato tubers through bruises in the skin occasioned by the handling of the potato crop in harvesting. A type of storage dryrot known as "powdery dryrot" and ascribed to the parasite *Fusarium trichothecioides* Wollenw. is apparently restricted to the arid and semiarid sections of the western part of the United States. Undoubtedly rots due to other causes also occur, but powdery dryrot is the only storage-rot causing enough damage to be of any great economic importance in the irrigated West. It would be difficult to arrive at any definite statement of the losses entailed by this disease, but it is known that they have been enormous. In several cellars visited, the writer estimated the losses caused by partial and total decay of the tubers to be from 30 to 50 per cent. Reports from farmers show that in some cases the losses have been much greater. This storage dryrot may be described as an external dryrot proceeding from bruises in the skin of the tuber. The decayed portion usually presents a wrinkled, sunken appearance, and in advanced stages may show a pinkish white growth of the fungus (Pl. CVIII, fig. 1, 2). The decayed tissue presents various shades of color from nearly black to light brown, the most characteristic color being sepia brown. Internal cavities partially filled with the mycelium and spores of the fungus are frequently found in decayed tubers (Pl. CVIII, fig. 3).

The first description of this disease was made in 1912 by Jamieson and Wollenweber,<sup>1</sup> who demonstrated that the rot was caused by a species of *Fusarium* which they called "*Fusarium trichothecioides* Wollenw." One year later Wilcox, Link, and Pool<sup>2</sup> described a dryrot of potato tubers in Nebraska, ascribing it to a species of *Fusarium* which they called "*Fusarium tuberivorum*." This fungus has since been demonstrated by Wollen-

<sup>1</sup> Jamieson, Clara O., and Wollenweber, H. W. An external dry rot of potato tubers caused by *Fusarium trichothecioides* Wollenw. *In* Jour. Wash. Acad. Sci., v. 2, no. 6, p. 146-152, 1 fig. 1912.

<sup>2</sup> Wilcox, E. M., Link, G. K. K., and Pool, Venus W. A dry rot of the Irish potato tuber. *Nebr. Agr. Exp. Sta. Research Bul.* 7, 88 p., 15 fig., 28 pl. (1 col.). 1913. Bibliography, p. 85-88.

weber<sup>1</sup> and by Carpenter<sup>2</sup> to be identical with *F. trichothecioides* Wollenw. Wilcox, Link, and Pool<sup>3</sup> found that their fungus was incapable of attacking the tubers through the eyes or lenticels and that it was incapable of attacking the growing plants. Jamieson and Wollenweber,<sup>3</sup> however, working with *F. trichothecioides* obtained from western potatoes, found that *F. trichothecioides* was capable of attacking the growing plant, and they also obtained infections through the unbroken skin of the tuber by rubbing the inoculum over the surface. Their results were obtained under the extremely humid conditions of the Department greenhouses at Washington, D. C.

Working with the same fungus, the writer was unable under the western field or laboratory conditions to produce infection through the unbroken skin of the potato tuber or to produce an infection in any part of a growing potato plant. His results agree in the main with those obtained by Wilcox, Link, and Pool,<sup>4</sup> thus further establishing the identity of *F. trichothecioides* with the so-called *F. tuberivorum*.

Preliminary work on this potato-tuber disease was begun in 1912, when the author was connected with the Agricultural Experiment Station of the University of Idaho. During the fall of 1912 and the spring of 1913 potato shippers reported heavy losses in carload lots of potatoes en route from Idaho and Utah to eastern and southern markets. Examination of infected tubers from such cars invariably revealed the presence of *F. trichothecioides*. In the fall of 1913 the writer was enabled to begin a study of storage conditions of potatoes. This study was continued up to the spring of 1916. It is safe to say that powdery dryrot can be found in every potato storage cellar in the areas covered by the author's investigations. However, when storage conditions were found to be good, losses were being reduced to a minimum.

During the whole course of the investigations, experiments leading to a further knowledge of the relationship of the fungus to the disease, as well as practical experiments leading to its control, were carried on. These experiments were conducted in part in the laboratories in Washington, D. C., and in part in the field, laboratory, and storage cellar of the Jerome Experiment Station, Jerome, Idaho. The work was further supplemented by the planting of seed plots in various places in southern Idaho. The results of these experiments, as set forth in this paper, are believed to be of fundamental scientific importance, since they throw more light on the relationship of the fungus to the disease and demonstrate a fairly successful method of control.

<sup>1</sup> Wollenweber, H. W. *Ramularia*, *Myeosphaerella*, *Nectria*, *Calonectria*. Eine morphologisch pathologische Studie zur Abgrenzung von pilzgruppen mit cylindrischen und sichelförmigen Konidienformen. *In* *Phytopathology*, v. 3, no. 4, p. 206. 1913.

<sup>2</sup> Carpenter, C. W. Some potato tuber-rots caused by species of *Fusarium*. *In* *Jour. Agr. Research*, v. 5, no. 5, p. 183-210, pl. A-B (col.), 14-19. 1915. Literature cited, p. 208-209.

<sup>3</sup> Jamieson, Clara O., and Wollenweber. *Op. cit.*

<sup>4</sup> Wilcox, E. M., Link, G. K. K., and Pool, Venus W. *Op. cit.*

PARASITISM OF *FUSARIUM TRICHOHECIOIDES*

To determine the parasitism of *F. trichothecioides*, several attempts were made to induce infection in various parts of growing plants and in mature tubers.

1. In the fall of 1913 half-bushel lots of unbruised tubers were obtained of each of the following varieties: Burbank, Idaho Rural, Early Rose, Peoples, Improved Peachblow, Netted Gem, and Pearl. All of the tubers selected were free from any external evidence of disease and were disinfected by dipping in a solution of formaldehyde (1:240). Each tuber in one half-bushel lot of each variety was bruised with a sterile knife and the bruised surface dipped in a suspension of the spores of the fungus. Each tuber in another half-bushel lot of each variety was first carefully examined to make sure that its skin was wholly sound and was then dipped in a suspension of the spores of the fungus. Checks of the same quantity of tubers of each variety were prepared in the same manner, except that the tubers, whether sound or bruised, were dipped in sterile water. Each lot was then placed in a sterilized canvas sack. To insure a high degree of humidity each sack was sprayed with sterile water. The sacks were then stored in one corner of the cellar and covered with canvas. The tubers were not examined until the following May, or about seven months after having been placed in storage. At the time of examination all tubers had sprouted, showing that temperature conditions, at least during the latter part of the storage period, had been ideal for the development of the rot. Every inoculated, bruised tuber showed infection, each bruised, inoculated tuber being from one-eighth to three-fourths decayed. None of the inoculated sound tubers showed any infection. In the checks there was a slight amount of decay in many of the bruised tubers, though they had not been inoculated; but all of the sound tubers of the checks remained sound throughout the storage period.

2. In the fall of 1914 further attempts were made to infect potato tubers with *F. trichothecioides* through the unbroken skin. The following varieties were employed: Improved Peachblow, Idaho Rural, Netted Gem, Peoples, and Pearl. Fifty sound tubers of each variety were first disinfected in a formaldehyde solution (1:240), dried, and then dipped in a spore suspension of the fungus. Fifty tubers of each variety were disinfected in the same manner, bruised with a sterile knife, and dipped in a suspension of the spores of the fungus. Each lot of tubers was then placed in a disinfected canvas sack. The potatoes were first stored in the laboratory culture room, where the temperature was very favorable to the development of the decay. A high humidity was maintained in the culture room by spraying the walls with sterile water. After a month the potatoes were removed from the culture room to the potato storage cellar, where they remained until spring. An examination of

the potatoes was made in April, 1915. None of the unbruised tubers showed any signs of infection, but infection was present in each of the bruised tubers.

3. In 1914, attempts to artificially infect growing potato plants with *F. trichothecioides* were made as follows:

a. One hundred apparently healthy Idaho Rural plants were selected, and the stem of each was punctured at the crown with a needle inoculated with the spores of the fungus. As a check, twenty-five apparently healthy plants of the same variety were selected and their stems punctured at the crown with a sterile needle.

b. One hundred apparently healthy Idaho Rural plants were selected. The soil was removed to expose one tuber under each plant. One tuber under each plant was punctured with a needle inoculated with the spores of the fungus. As a check, twenty-five apparently healthy plants of the same variety were selected and the soil removed to expose one tuber under each plant, which was then punctured with a sterile needle.

c. One hundred apparently healthy Idaho Rural plants were selected and the soil removed to expose one tuber under each plant. The stolon of one tuber under each plant was then punctured with a needle inoculated with the spores of the fungus. As a check, twenty-five apparently healthy plants of the same variety were selected and the soil removed to expose one tuber under each plant. One tuber stolon under each plant was then punctured with a sterile needle.

An examination was made one month later. No evidence of infection could be found in the proximity of the punctures in the stems, the tubers, or the tuber stolons. The punctures made were so large that they could be seen easily in each case, but apparently they had healed over. The checks presented the same appearance.

As the foregoing inoculations of growing plants had been made rather late in the season (August 21), it was thought that the failure to develop any infection might have been due to the late date on which the inoculations were made. Therefore the attempts were repeated in 1915 as follows:

1. Fifty Netted Gem tubers which had been inoculated with *F. trichothecioides* were kept for several days in moist chambers at temperatures favorable for the development of the fungus. On June 4, when the decay was well advanced, the fifty tubers were planted in a Station plot in an attempt to infect the growing plants through the seed pieces. A similar number of hills of the Netted Gem variety were planted with disease-free seed pieces as a check. The plants were examined from time to time during the season, cultures being made whenever any evidence of disease appeared, but *F. trichothecioides* was never obtained. The plot was dug on September 15, when all stems and tubers were examined for evidence of disease. There was no evidence of decay in the harvested tubers, and the stems of the plants were usually white and clean. Six

plants out of the fifty which resulted from the planting of the inoculated seed pieces showed vascular infection, but *F. trichothecioides* could not be recovered.

2. Twenty-five Idaho Rural tubers, first disinfected by dipping in formaldehyde, were placed in moist chambers and allowed to develop sprouts. On July 10, when the sprouts were from one-eighth to one-half inch long, they were sprayed with a spore suspension of the fungus. As a check, twenty-five Idaho Rural tubers were treated in the same manner, but the sprouts were sprayed with sterile water. After a little more than a month each sprout was carefully examined. No evidence of infection was found either in the sprouts sprayed with the spore suspension or in the checks.

3. On July 11 further attempts to infect growing potato plants were made as follows:

a. Ten apparently healthy Idaho Rural plants were selected. The soil was removed to expose as many of the tubers as possible without disturbing their position. In all, twenty-five tubers were uncovered and punctured with a needle inoculated with the spores of the fungus, after which the soil was replaced. As a check, a similar number of plants of the same variety were selected and twenty-five tubers punctured with a sterile needle. This experiment was duplicated with Netted Gems.

b. Ten apparently healthy Idaho Rural plants were selected. The soil was removed to expose as many of the tubers as possible without disturbing their position. Twenty-five tubers thus uncovered were sprayed with a suspension of the spores of the fungus, after which the soil was replaced. To prevent the rapid drying off of the sprayed tubers, the soil when replaced was moistened. As a check, ten other apparently healthy Idaho Rural plants were selected and twenty-five tubers sprayed with sterile water. The soil was moistened upon being replaced. This experiment was duplicated with Netted Gems.

c. Ten apparently healthy Idaho Rural plants were selected. The soil was removed to expose as many of the tubers with their stolons as possible without disturbing their position. The stolons of twenty-five tubers thus uncovered were then punctured with a needle inoculated with the spores of the fungus, after which the soil was replaced, care being exercised to place moist soil next to the inoculations. As a check, ten other plants of the same variety were selected and twenty-five tuber stolons punctured with a sterile needle, after which the soil was replaced. This experiment was duplicated with Netted Gems.

d. Ten apparently healthy Idaho Rural plants were selected and the stem of each plant punctured at the crown with a needle inoculated with the spores of the fungus. As a check the stems of ten plants were punctured with a sterile needle. This experiment was duplicated with Netted Gems.

e. The leaves of ten apparently healthy Idaho Rural plants were sprayed with a spore suspension of the fungus. As a check, the leaves of ten apparently healthy plants were sprayed with sterile water. This experiment was duplicated with Netted Gems.

In the fall a careful examination was made of each plant and tuber. Not the slightest trace of infection that could be ascribed to *F. trichothecioides* could be found, though cultures were made from suspicious-looking stem lesions and tuber discolorations. The punctures in the tubers and stolons had healed over, leaving only the slightest scars as evidence. The punctures in the stems could be found by very careful scrutiny, but were entirely healed over. There was neither internal nor external evidence of disease in the neighborhood of the punctures, whether in stems, tubers, or tuber stolons. No disease appeared in the foliage or stems as a result of spraying with the spore suspension.

The results of the attempts to induce infection in growing potato plants were such as might have been expected after several years' search in commercial fields for evidence of disease which could be attributed to this organism. Several hundred cultures have been made from diseased parts of growing potato plants. Out of these attempts, *F. trichothecioides* has been obtained but 13 times, twice from Netted Gem tubers infected with jelly-end rot and 11 times from potato stems infected with footrot. In the footrot cultures, *F. trichothecioides* was associated with other species of *Fusarium*, including *F. radicum* and *F. oxysporum*, as well as other fungi. It is not likely that *F. trichothecioides* attacked the growing stem, but rather it is probable that it entered as a secondary organism after the attacks of other fungi or bacteria. The writer has shown in another paper<sup>1</sup> that jelly-end rot does not develop at temperatures below 10° C.; therefore *F. trichothecioides* is eliminated as one of the contributing causes of this fieldrot, since, if *F. trichothecioides* were generally present in tubers infected with jelly-end rot, such tubers when placed in storage would continue to decay at temperatures as low as 4° (see pages 825 to 827). *F. trichothecioides* has never been obtained from any other fieldrot. In commercial storage cellars, unbruised tubers have never been found infected by *F. trichothecioides*; on the other hand, the majority of bruised tubers in storage show more or less decay from this cause.

#### EFFECT OF PLANTING SEED INFECTED BY DRYROT

Poor stands of potatoes have been observed from year to year in many potato fields in southern Idaho. In many cases it was impossible to say whether the poor stand was due to irregularity in planting or to poor seed. In some cases, however, the only explanation that could be made

<sup>1</sup> Pratt, O. A. A western fieldrot of the Irish potato tuber caused by *Fusarium radicum*. *In Jour. Agr. Research*, v. 6, no. 9, p. 297-310, pl. 34-37. 1916.

was the known fact that seed infected with dryrot had been planted. To determine the effect on the stand of planting seed infected with dryrot, several plots of potatoes were planted as follows:

Plot 1.—Each seed piece showed at least one healthy eye, but was almost wholly decayed. The variety planted was Idaho Rural.

Plot 2.—Each seed piece showed a pocket of dryrot at least half an inch in diameter and about as deep as wide. The variety planted was Idaho Rural.

Plot 3.—This plot was planted with seed of the same character as that used in plot 1, except that the variety was Netted Gem.

Plot 4.—This plot was as nearly as possible a duplicate of plot 2, except that the variety planted was Netted Gem.

Two check plots were also planted, one of Netted Gem and one of Idaho Rurals. In each of the check plots only seed entirely free from disease was used. The plots were planted on the grounds of the experiment station at Jerome, Idaho. Table I shows the stand which resulted.

TABLE I.—Percentage of stand of potatoes in plots infected with powdery dryrot

Plot No.	Variety.	Percentage of stand.	
		Four weeks after planting.	Six weeks after planting.
1.....	Idaho Rural.....	72	82
2.....	do.....	98	100
3.....	Netted Gem.....	70	85
4.....	do.....	99	100
Check.....	do.....	100	100
Do.....	Idaho Rural.....	100	100

In plots 1 and 3, in which the seed planted was nearly totally decayed, the stand never exceeded 82 per cent of the Idaho Rural nor 85 per cent of the Netted Gem. The plants in these two plots were much slower in coming up than those in the check plots or in plots 2 and 4. One month after planting, all the seed in the check plots had produced plants larger and stronger than those in plots 1 and 3, but no difference was observed between the plants in the check plots and those in plots 2 and 4. Although the stand in plots 2 and 4 was not quite perfect at the end of the first month, the stragglers soon appeared, and a perfect stand resulted. The results of these experiments specifically agreed with the observations in commercial fields. It is believed that had the wet weather of the early spring continued throughout the month of June, a much smaller percentage of the seed would have produced plants. The plots were carefully watched throughout the growing season; but after the plants had thoroughly established themselves, there was little or no difference between the plants in the diseased plots and those in the

check plots. The plants in plots 1 and 3 eventually became as strong and vigorous as those in plots 2 and 4 and in the check plots. At harvest time 100 hills from each plot were dug and the tubers carefully examined for the evidence of disease. Table II shows the percentage of disease present in the tubers at harvest time.

TABLE II.—Percentage of disease present in potato tubers at harvest time

Plot No.	Variety.	Scab.	Rhizoctonia scab.	Vascular infection.	Powdery dryrot.
1.....	Idaho Rural.....	2	○	36	○
2.....	do.....	○	○	38	○
3.....	Netted Gem.....	○	○	32	○
4.....	do.....	○	○	33	○
Check.....	do.....	○	○	21	○
Check.....	Idaho Rural.....	○	○	59	○

It is evident from the results that dryrot infection in the seed does not in any way influence the amount of disease in the product. No dryrot appeared in any of the plots at harvest time. The percentage of vascular infection was higher in the case of one of the check plots and lower in the other than in the diseased seed plots. A large number of cultures were made from the discolored vascular tissues of the tubers from all of the plots, but the fungus *F. trichothecioides* was never once obtained.

#### SOURCE OF THE ORGANISM CAUSING POWDERY DRYROT

It was evident that the organism causing the decay must be present in the soil particles clinging to the surface of the tubers when harvested, but whether *F. trichothecioides* was present in the soil prior to the planting of the potatoes or was introduced with the seed was not known. It was thought that the latter might be the case. Accordingly plots of potatoes in which all the seed was entirely free from disease and had been disinfected for 1½ hours in a solution of mercuric chlorid (1:1,000) were planted on both raw desertland and lands previously in alfalfa. Check plots were also planted in which each seed piece was well infected with the rot.

At harvest time samples of potatoes from each of the plots were placed in sterilized tin boxes and put in storage at temperatures favorable for the development of the rot. Each tin box used in the experiment was first wrapped in heavy paper and sterilized for three hours in the oven at a temperature of 160° C. To secure the samples of potatoes, the sterile boxes were taken to the field and a hill or more of potatoes dug with a trowel which had first been sterilized. The tubers were then bruised with the same trowel, the box opened, and the potatoes put into the box with a little of the moist soil in which the tubers had been growing, in order to insure proper moisture conditions within the box. The box was



then closed, wrapped, and stored. Eight of these samples were obtained: Two of Netted Gem and one of Idaho Rural from desert land plots, and one of the Netted Gem and two of Idaho Rural from alfalfa-land plots. The remaining two samples were taken from the check plots which were planted on alfalfa land. One was a sample of the Netted Gem variety, and the other was an Idaho Rural. Two months after storing the samples, the boxes were opened and the tubers examined. Every tuber in each of the eight boxes showed at least slight signs of decay, and some showed deep infection pockets of dryrot.

Isolations were made from the decayed portions of the tubers and the presence of the organism determined. Not a single culture gave negative results. It is apparent, therefore, that *F. trichothecioides* is at the present time well distributed in desert soils, as well as in those previously in cultivation, and is not necessarily introduced on the seed.

#### RELATIONSHIP OF TEMPERATURE TO THE DEVELOPMENT OF POWDERY DRYROT

The experiments to determine the relationship of temperature to the development of powdery dryrot were carried on in the laboratories in Washington and in the cold-storage rooms of a Washington cold-storage plant. In these experiments potatoes of the following varieties were used: Idaho Rural, Netted Gem, Peoples, Pearl, Burbank, and Improved Peachblow. Three different experiments were undertaken.

1. Tubers of each of the above varieties were first washed and disinfected by fumigating with formaldehyde gas. Two methods of inoculation were employed. The first method consisted in cutting off the stem end of the tuber and dipping the cut surface into a spore suspension of *F. trichothecioides*. The second method consisted in inoculating the tubers by puncturing the skin with a needle inoculated with the spores of the fungus. In both cases the inoculated tubers were wrapped separately in sterile paper. Tubers inoculated by each of these methods were placed in the incubators and in the incubator room. Checks were prepared in the same manner except that the tubers in one case were dipped in sterile water and in the other case were punctured with a sterile needle.

2. In the second experiment sterile blocks were cut from tubers from each of the varieties named. These sterile blocks were placed in sterile culture tubes and allowed to incubate for several days in order to insure their sterility, after which they were inoculated with the fungus and placed in the incubators and in the incubator room.

3. In the third experiment half-bushel lots of each of the varieties above named were first washed and disinfected by fumigating with formaldehyde gas. Each tuber was then cut across the stem end and the cut surface dipped in a spore suspension of the fungus, after which they

were wrapped separately in sterile paper. Each half-bushel lot was then placed in a tin box which had first been sterilized. Half-bushel lots of each variety thus prepared were placed in cold storage at temperatures of 0° and 1.1° C. Half-bushel lots of each variety were prepared in the same manner and placed in the incubator room as a check. Check lots in which the tubers were treated in the same manner but not inoculated were also placed in the incubator room and in cold storage at temperatures of 0° and 1.1° C. Table III gives the results of these tuber inoculations under the different storage conditions, showing the temperatures of the incubator chambers, the incubator room, the rooms in the cold-storage plant during the period of storage, and the condition of the inoculated tubers at the end of the storage period. In Table III the incubator chambers are designated by numbers 1 to 10 and the cold-storage rooms as A and B. All of the uninoculated checks remained sound.

TABLE III.—Results of potato-tuber inoculations under different storage conditions

Incubator chamber No.	Temperatures during period of storage.			Condition of inoculated tubers at termination of storage period.
	Minimum.	Maximum.	Average.	
	° C.	° C.	° C.	
1.....	0.2	2.1	0.8	Sound.
2.....	4.0	7.3	4.2	Very slight decay.
3.....	4.2	10.2	7.6	One-third to two-thirds decayed.
4.....	6.3	12.5	8.9	Nearly total decay.
5.....	8.9	14.4	12.0	Total decay.
6.....	10.0	17.0	14.7	Do.
7.....	11.0	19.0	17.0	Do.
8.....	14.0	21.8	18.0	Do.
9.....	15.0	25.1	19.0	Do.
10.....	13.8	22.5	19.9	Do.
Incubator room.....	19.0	26.5	25.0	Do.
A.....	0	0	0	Sound.
B.....	1.1	1.1	1.1	Do.

It is evident from the results obtained that powdery dryrot will not develop at temperatures below 2° C. At temperatures ranging from 2° to 4° (35° to 40° F.) the amount of decay will be slight, especially if the storage rooms are kept fairly dry and well ventilated.

#### INFLUENCE OF HUMIDITY ON THE DEVELOPMENT OF POWDERY DRYROT IN STORAGE

It has often been observed in storage cellars which were comparatively dry and well ventilated that the losses from powdery dryrot were much less than in damp, poorly ventilated cellars. The writer has been in cellars where practically every bruised tuber was from one-third to nearly totally decayed. Such cellars have invariably been exceedingly damp

and poorly ventilated. He has been in other cellars where the bruised tubers showed only an incipient rot, the decay usually extending inward from the bruised surface for less than one-fourth of an inch. Such cellars have invariably been very dry and well ventilated. It is to be regretted that there has been no opportunity to obtain the percentages of atmospheric humidity most favorable to the development of the rot. However, a preliminary study of the effect of humidity on powdery dryrot development was undertaken in the spring of 1915.

In the month of April, owing to the fact that heavy rains had been falling, the storage cellar of the Jerome Experiment Station was in a comparatively damp condition, the doors having been open for a considerable portion of the time to allow workmen to enter. At the same time the cellar under the Station laboratory building was being kept in a comparatively dry condition, while the air of the laboratory itself was very dry, owing to the fact that fire was being constantly maintained in the stove. One hundred and fifty Netted Gem tubers inoculated with *F. trichothecioides* were allowed to remain for several days in moist chambers until the fungus had well established itself, after which fifty tubers were removed to the potato storage cellar; fifty of the tubers were put in the cellar of the laboratory building, while the remaining fifty tubers were exposed to the dry air of the laboratory room. After six weeks the potatoes were examined. Though the temperature had been very favorable for the development of the rot, the fifty tubers left in the dry laboratory room showed no apparent advance in the decay from the time they had been removed from the moist chambers. Those in the laboratory cellar showed but a very slight advance in the decay, while those in the storage cellar showed well-defined pockets of dryrot, each tuber being from one-eighth to one-fourth decayed. This preliminary experiment shows that the drier the atmosphere the less will be the decay in storage from this cause.

#### DISINFECTION OF POTATO STOCK BEFORE STORING

In order to learn whether the progress of powdery dryrot in storage could be inhibited by disinfecting the potatoes before storage, the following experiments were set up. Both bruised and sound tubers were employed. The bruised ones had been injured in the field during the process of digging. Fumigation with formaldehyde gas was the method of disinfection used. The potatoes were fumigated in an air-tight room at a temperature of about 60° F. To produce the formaldehyde fumes the following formula was employed: Formaldehyde (40 per cent), 3 pints; potassium permanganate, 23 ounces for each 1,000 cubic feet of space. The potatoes were arranged in two lots as follows:

Lot 1 in trays, each tray holding about 50 pounds of potatoes. One tray each of bruised tubers of Early Rose, Improved Peachblow, Peoples, Netted Gem, and Pearl, and three trays of bruised tubers of Idaho Rural; also a similar number of trays of sound tubers of each variety.

Lot 2 in sacks, each holding about 100 pounds. One sack each of bruised tubers of Early Rose, Improved Peachblow, Peoples, Netted Gem, and Pearl and two sacks of bruised tubers of Idaho Rural; also a similar number of sound tubers of each variety. The potatoes were fumigated for 24 hours and then placed in storage. As a check, a similar number of trays and sacks of bruised tubers of each variety and a similar number of trays and sacks of sound tubers of each variety were put in storage without fumigation. The period of storage was from November 1, 1913, to May 10, 1914. The cellar was well ventilated and comparatively dry. The temperature throughout the storage period ranged from 0° as a minimum to 7.8° C. as a maximum.

A careful examination of the potatoes at the end of the storage period revealed the fact that a slight amount of decay had taken place in all of the bruised tubers, whether fumigated or unfumigated. Cultures were made from a large number of infected tubers, and the fungus *F. trichothecioides* was obtained. No apparent difference was noted between the fumigated and the unfumigated lots, and there was no decay in any of the unbruised tubers, whether fumigated or not. The unfumigated potatoes, however, sound or bruised, presented a much better appearance than the fumigated potatoes, owing to the injuries in the form of sunken spots which appeared on most of the fumigated tubers caused by the action of the formaldehyde fumes.

In the fall of 1915, other experiments to control powdery dryrot by disinfecting prior to storage were undertaken. On September 27, Idaho Rural tubers were dug for the experiment. One-half of the tubers were bruised in the field with the digging fork. Bruised and sound tubers were sacked separately. Twenty-five sacks of bruised tubers and twenty-five sacks of sound tubers were employed in the experiments. Each sack contained about 40 pounds of potatoes. The methods of disinfection were as follows: (1) The formaldehyde dip (1 pint of 40 per cent formaldehyde to 30 gallons of water). (2) The mercuric-chlorid dip (4 ounces of mercuric chlorid to 30 gallons of water). (3) Dusting with flowers of sulphur. (4) Dusting with air-slaked lime. The formaldehyde and mercuric-chlorid solutions were made up fresh for each disinfection.

The potatoes dug were divided into five lots, each lot consisting of five sacks of bruised tubers and five sacks of sound tubers. On September 27, a few minutes after digging, one sack of bruised tubers and one sack of sound tubers were placed in storage without disinfection. One sack each of bruised and sound tubers was dipped for two hours in the formaldehyde solution and then dried and put in storage. One sack each of bruised and sound tubers was dipped for two hours in the mercuric-chlorid solution, dried, and put in storage, one sack each of bruised and sound tubers was dusted with flowers of sulphur and put

in storage, and one sack each of bruised and sound tubers was dusted with lime and put in storage. On September 28, twenty-four hours after digging, the second lot, consisting of five sacks each of bruised and sound tubers, was treated in the same manner as that of the previous day and placed in storage. On September 29, forty-eight hours after digging, the third lot, consisting of five sacks each of bruised and sound tubers, was treated in the same manner as the first and second lots and placed in storage. On September 30, seventy-two hours after digging, the fourth lot, consisting of five sacks each of bruised and sound tubers, was treated in the same manner as lots 1, 2, and 3 on previous days and placed in storage. On October 1, ninety-six hours after digging, the fifth lot, consisting of five sacks each of bruised and sound tubers, was treated in the same manner as those on the previous days and was put in storage. In each case the disinfected potatoes were stored in the sacks in which they were disinfected. In order to give the experiment a severe test all five lots were stored for about six weeks in the anteroom of the storage cellar, where temperature and moisture conditions were favorable for dryrot development, after which they were transferred to the storage cellar proper.

On April 1, 2, and 3, 1916, examination of the potatoes was made. Each tuber was carefully examined to determine the presence or absence of decay. Wherever decay occurred, typical specimens were taken to the laboratory, and the presence of *F. trichothecioides* was determined by means of artificial cultures. All of the unbruised tubers, whether disinfected or not, were still wholly sound. The bruised tubers which were not disinfected presented essentially the same appearance in all lots. Those with deep bruises were usually from one-third to totally decayed. Those with shallow bruises in some cases showed no decay, but the majority showed at least slight decay. By "deep bruises" are meant those which penetrated the tuber tissue far enough to be partly closed up and covered over when the digging instrument was withdrawn; by "shallow bruises," those which were only skin deep, or which presented a comparatively clean cut surface. The condition of the disinfected, bruised tubers at the end of the storage period is shown in Table IV.

TABLE IV.—Condition of the disinfected, bruised potato tubers at the end of the storage period

Time of disinfection.	Condition after disinfection with—			
	Mercuric chlorid.	Formaldehyde.	Lime.	Sulphur.
Immediately after digging.	No decay. . . . .	No decay. . . . .	Decay in most tubers with deep bruises. Shallow bruises healed over.	Presented the same appearance as those dusted with lime.
24 hours after digging.	.....do. ....	.....do. ....	Presented the same appearance as those disinfected immediately after digging.	Do.
48 hours after digging.	Very slight decay in most tubers with deep bruises. Shallow bruises healed over.	All tubers with deep bruises from one-third to one-half decayed. Tubers with shallow bruises showing slight or no decay.	.....do. ....	Do.
72 hours after digging.	Well - established dryrot in all tubers with deep bruises. Shallow bruises healed over.	Presented the same appearance as those disinfected 48 hours after digging.	.....do. ....	Do.
96 hours after digging.	All tubers with deep bruises from one-quarter to one-third decayed. Slight decay proceeding from shallow bruises.	A few tubers totally decayed. Balance presented the same appearance as those disinfected 48 hours after digging.	.....do. ....	Do.

It is evident from the results obtained by disinfecting potato stock prior to storage that it is possible to check the disease effectively, provided the disinfecting is done within 24 hours after digging. The solution of mercuric chlorid, which was the most effective, was fairly efficient 48 hours after digging. The formaldehyde solution gave the next best results and was thoroughly effective 24 hours after digging. It was of little or no value when applied from 48 to 96 hours after digging. There was little difference to be observed between the lots dusted with lime and those dusted with sulphur. Wherever the tuber bruises were of such a character that the lime or sulphur could reach and cover the bruised

surface, no decay occurred. The lime and sulphur dust did not always reach the deeper bruises and therefore was not effective in such cases. Disinfecting potatoes with mercuric chlorid or formaldehyde prior to storage should be of value when it is necessary to store seed potatoes in a poorly ventilated or improperly cooled storage cellar. Lime and sulphur are not recommended.

#### SUMMARY

(1) Powdery dryrot, caused by *Fusarium trichothecioides*, is the most important storage-rot affecting potatoes in the irrigated West.

(2) *F. trichothecioides* under ordinary western field conditions does not attack any part of the growing potato plant. Potatoes in storage are attacked only through bruises.

(3) Planting badly infected seed potatoes greatly reduces the stand. A slight amount of infection in the seed piece does not cause any serious loss.

(4) The causal organism is at the present time apparently well distributed throughout western desert soils.

(5) *F. trichothecioides* does not develop at a temperature below 2° C. No loss from powdery dryrot occurs when the storage house is kept at this temperature, or lower. In a dry, well-ventilated storage house losses will be very slight at temperatures from 2° to 4° C. (35° to 40° F.).

(6) Where it is necessary to store seed potatoes in a poorly ventilated or improperly cooled storage house, the disease may be effectively checked by disinfecting the stock, prior to storage, with a solution of mercuric chlorid or formaldehyde, provided the disinfecting is done immediately, or within 24 hours after digging.

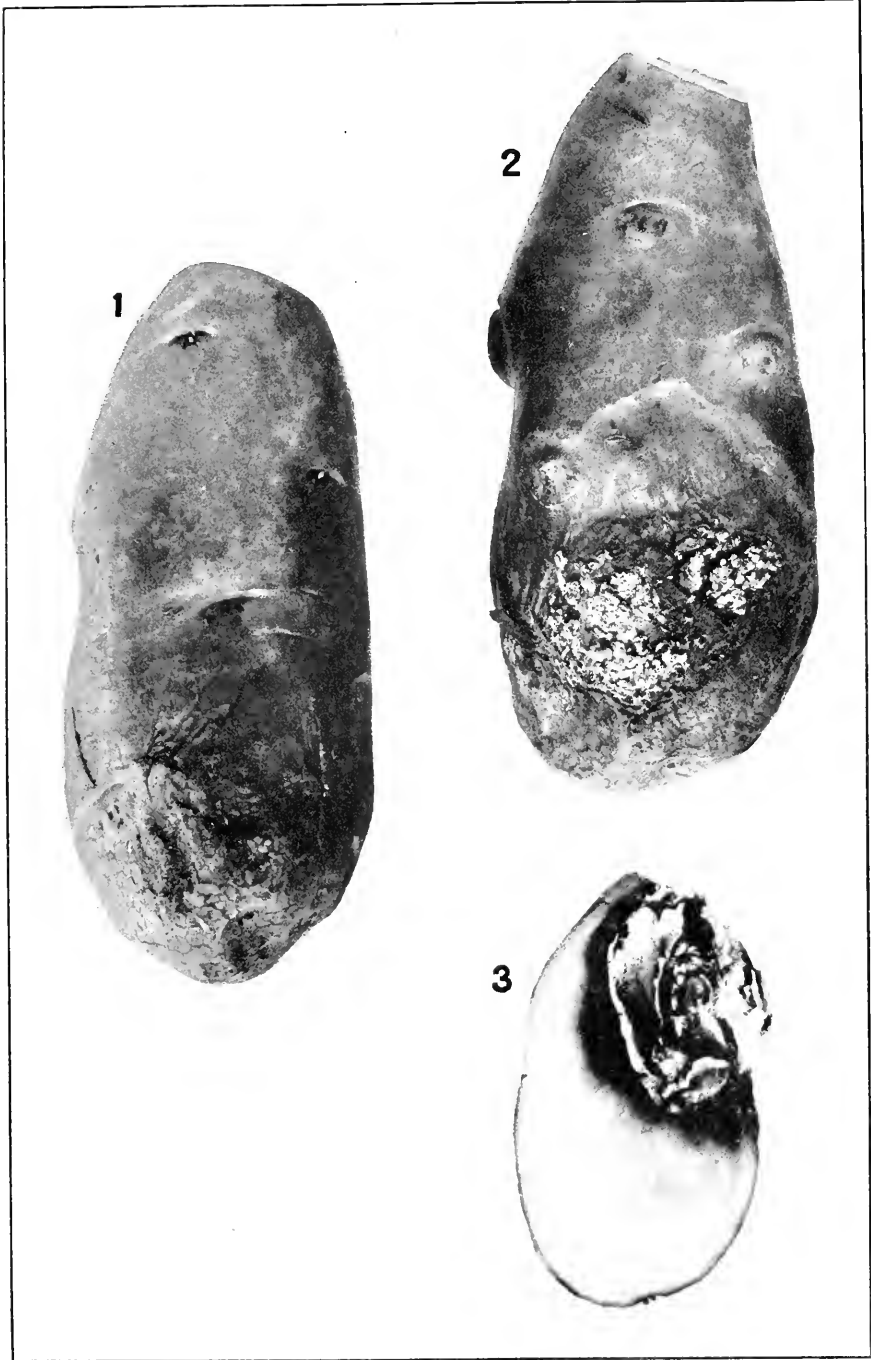
PLATE CVIII

Fig. 1.—A potato tuber infected with powdery dryrot, showing the wrinkled condition of skin due to the decay of underlying tissues.

Fig. 2.—A potato tuber infected with powdery dryrot: Advanced stage. Note the presence of *Fusarium trichothecioides* on the surface of the decayed portion of the tuber.

Fig. 3.—Section through a potato tuber infected with powdery dryrot, showing the internal cavities filled with the mycelium and the spores of the fungus.







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## USE OF THE MOISTURE EQUIVALENT FOR THE INDIRECT DETERMINATION OF THE HYGROSCOPIC COEFFICIENT

By FREDERICK J. ALWAY, *Chief, Division of Soils, Agricultural Experiment Station of the University of Minnesota*, and JOUETTE C. RUSSEL, *Professor of Chemistry and Physics, McPherson College, Kansas*

### INTRODUCTION

The maximum amount of soil water available for growth and for the maintenance of life in the case of ordinary crop plants appears to be approximately equal to the free water—the difference between the total amount of water and the hygroscopic coefficient—in those portions of the soil and the subsoil occupied by the roots (1, p. 121).<sup>1</sup> The hygroscopic coefficient (8, p. x; 10, p. 243) expresses the percentage of moisture contained in a soil which, in an air-dry condition, has been brought into a saturated atmosphere, kept at a constant temperature, and allowed to remain until in approximate equilibrium with this atmosphere.

Hilgard's method for the direct determination (10, p. 243; 11, p. 17) of the hygroscopic coefficient requires provision for the maintenance of a constant temperature in the room in which the absorption boxes are placed and also presents difficulties in insuring the actual saturation of the atmosphere in these boxes. Accordingly, any indirect method which gives results in satisfactory accord with those obtained by direct determination and at the same time requires only apparatus which is less inconvenient, either of installation or of operation, will prove useful.

Briggs and Shantz (7, p. 73) have recently proposed several indirect methods, and to the consideration of the reliability of one of these the present paper is devoted. These authors derived formulas for the indirect determination of what they designate the "wilting coefficient," defined as the moisture remaining in the soil in immediate contact with the roots when the permanent wilting of a plant occurs, from the moisture equivalent (6, p. 140; 4, p. 276), from the maximum water capacity as

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<sup>1</sup> Reference is made by number to "Literature cited," p. 845.

defined by Hilgard (10, p. 256), and from the mechanical analysis. Subsidiary formulas for the indirect determination of the hygroscopic coefficient, following as a result of the interrelationships thus established, they report (7, p. 73) as follows:

$$\text{Hygroscopic coefficient} = \begin{cases} \text{Wilting coefficient} \times 0.68. \\ \text{Moisture equivalent} \times 0.37. \\ (\text{Maximum water capacity} - 21) \times 0.234. \\ (0.007 \text{ sand} + 0.082 \text{ silt} + 0.39 \text{ clay}). \end{cases}$$

As the mechanical analysis of a soil is a far more difficult and time-consuming operation than the determination of the hygroscopic coefficient, the latter could advantageously be calculated from the former only where this is already available, as, for example, in the reports of soil surveys. Even then there is a probability of introducing serious errors. Thus, with a series of loess soils (3, p. 411) it has recently been shown that the values for the hygroscopic coefficient calculated by the Briggs-Shantz formula agree satisfactorily with those obtained by direct determination only in the case of those samples which carry the smallest proportion of very fine sand. However, by altering the values assigned the sands there was obtained the following modified formula, which was found applicable to all the loess soils investigated.

$$\text{Hygroscopic coefficient} = 0.005 \text{ coarser fractions} + 0.07 \text{ very fine sand} + 0.82 \text{ silt} + 0.39 \text{ clay.}$$

"Coarser fractions" is here used to designate all soil particles having a diameter greater than 0.10 mm.

The wilting coefficient also is so inconvenient of determination that, unless it has to be determined for some other purpose, it will not be used to calculate the hygroscopic coefficient.

In connection with field studies of available soil moisture on the Nebraska loess, of which only a few data (1, p. 118) have as yet been published, one of us had arrived at conclusions so widely at variance with those of Briggs and Shantz, who, in somewhat similar studies, had employed the wilting coefficient, either determined directly or calculated from the moisture equivalent, that we suspected the explanation might lie in the differences in the values of the hygroscopic coefficient obtained for similar soils by our respective methods. However, as no moisture-equivalent apparatus was at that time available for our use we were unable then to decide the question. Now, using 135 samples of which hygroscopic coefficient determinations had been made at the Nebraska Experiment Station, we have determined the moisture equivalents, thus obtaining a definite answer to the question.

Lipman and Waynick (12) have recently reported both the moisture equivalents and the hygroscopic coefficients of 27 soils, and from these the ratios may be calculated. In so far as we are aware, there are no published data except those in the two articles mentioned from which the

relationship of the moisture equivalent to the hygroscopic coefficient can be computed.

COMPUTATIONS FROM DATA OF BRIGGS AND SHANTZ

Briggs and Shantz have reported (7, p. 57-65) both the hygroscopic coefficients and the moisture equivalents in the case of 17 soils ranging in texture from a coarse sand with a hygroscopic coefficient of 0.5 to a clay loam with a value of 13.2. Their data, however, were not presented in such form as to show the concordance of the hygroscopic coefficients calculated from the moisture equivalents with those directly determined, and for this reason we consider it desirable to so present them (Table I).

TABLE I.—*Relation of the moisture equivalent to the hygroscopic coefficient shown by data of Briggs and Shantz*<sup>1</sup>

Soil.	Type of soil.	Moisture equivalent.	Hygroscopic coefficient.	Ratio of moisture equivalent to hygroscopic coefficient.	Hygroscopic coefficient calculated from moisture equivalent.	Departure of calculated from determined hygroscopic coefficient.
7	Coarse sand.....	1.6	0.5	3.20	0.6	0.1
2	Fine sand.....	4.7	1.5	3.13	1.7	.2
8	.....do.....	5.5	2.3	2.39	2.0	-.3
9	.....do.....	6.7	2.3	2.91	2.5	.2
3	Sandy loam.....	9.7	3.5	2.77	3.6	.1
10	.....do.....	11.9	4.4	2.70	4.4	.0
4	Fine sandy loam..	18.1	6.5	2.78	6.7	.2
12	Loam.....	18.9	7.8	2.42	7.0	-.8
A	Sandy loam.....	19.6	6.3	3.11	7.2	.9
B	Fine sandy loam..	19.9	6.6	3.01	7.3	.7
C	.....do.....	22.1	7.5	2.94	8.2	.7
5	Loam.....	25.0	9.8	2.55	9.2	-.6
D	.....do.....	27.0	9.6	2.81	10.0	.4
13	Clay loam.....	27.4	11.8	2.32	10.1	-1.7
14	.....do.....	29.3	13.2	2.22	10.8	-2.4
E	.....do.....	30.0	11.2	2.68	11.1	-.1
6	.....do.....	30.2	11.4	2.65	11.2	-.2
	Mean <sup>2</sup> .....			2.71		
	Maximum.....			3.11		
	Minimum.....			2.22		

<sup>1</sup> Derived from Briggs and Shantz (7, p. 57, 60, 65, Tables XVII, XIX, and XX).  
<sup>2</sup> Omitting 7 and 2.

Excepting the two sands, 1 and 2, the ratio varies from 3.11 to 2.22, a range of 40 per cent, reaching a maximum in the case of a sandy loam with a hygroscopic coefficient of 6.3 and a minimum in a clay loam with the coefficient 13.2. In the case of the latter the value calculated from the mean ratio, 2.71, differs by 2.4 from that obtained by direct determination. Two of the four clay-loam samples give concordant and two rather discordant results, the divergence in the case of the latter being similar to that obtained from the mechanical analysis of many of the loess soils (3, p. 411).

## COMPUTATIONS FROM DATA OF LIPMAN AND WAYNICK

Lipman and Waynick report (12, p. 8-9) both the hygroscopic coefficients and the moisture equivalents on 27 samples used in the well-known so-called Tri-State Soil Exchange Experiment. The ratios, which evidently they did not compare, we show in Table II. These data have an added interest in that they are from the laboratory of the late Dr. Hilgard, who introduced the determination of the hygroscopic coefficient (8, 9, 10).

TABLE II.—Relation of the moisture equivalent to the hygroscopic coefficient shown by the data of Lipman and Waynick

HYGROSCOPIC COEFFICIENT <sup>1</sup>									
Depth.	California soil.			Kansas soil.			Maryland soil.		
	In California.	In Kansas.	In Maryland.	In California.	In Kansas.	In Maryland.	In California.	In Kansas.	In Maryland.
<i>Feet.</i>									
1.....	8.55	8.29	6.68	12.12	10.74	11.00	5.97	5.15	4.69
2.....	8.67	7.69	8.44	12.42	12.38	11.68	6.82	5.82	7.66
3.....	8.98	8.68	9.04	11.28	10.54	11.18	8.87	6.75	9.23
Average.....	8.73	8.22	8.05	11.94	11.22	11.29	7.22	5.91	7.19

MOISTURE EQUIVALENT <sup>2</sup>									
1.....	24.09	22.32	22.67	32.61	29.63	29.80	23.62	23.67	21.92
2.....	22.81	22.20	20.32	33.33	30.78	31.14	24.26	26.02	19.37
3.....	24.03	24.24	23.53	30.21	27.57	29.40	29.17	29.16	27.38
Average.....	23.64	22.92	22.17	32.05	29.33	30.11	25.68	26.28	22.89

RATIO OF MOISTURE EQUIVALENT TO HYGROSCOPIC COEFFICIENT									
1.....	2.82	2.69	3.39	2.69	2.76	2.71	3.96	4.60	4.67
2.....	2.63	2.89	2.41	2.68	2.49	2.67	3.56	4.47	2.53
3.....	2.67	2.79	2.60	2.68	2.61	2.63	3.29	4.32	2.97
Average.....	2.71	2.79	2.80	2.68	2.62	2.67	3.60	4.46	3.39

<sup>1</sup> From Lipman and Waynick (12, p. 8, Table I). <sup>2</sup> From Lipman and Waynick (12, p. 9, Table II).

The average ratio for the 27 samples is 3.08, with a minimum of 2.41 and a maximum of 4.67, a range of 93 per cent. On inspection of Tables I and II it will be seen that for the Kansas soils the ratio varies only between 2.49 and 2.76, and for the California soils between 2.41 and 3.39, with an average for these 18 samples of 2.72, which is practically identical with the mean found by Briggs and Shantz—viz, 2.71.

In the case of the 9 samples of Maryland soils, the ratio varies from 2.53 to 4.67, with an average of 3.75. As none of the samples is to be considered lighter in texture than a loam or heavier than a clay loam, any ratio sufficiently accurate for ordinary purposes should apply to all of them.



## EXPERIMENTAL WORK

The moisture equivalents were determined according to Briggs and Shantz (7, p. 57), bringing the soils into equilibrium with a force 1,000 times that of gravity, using a centrifuge (6, p. 141) made according to specifications kindly furnished by Dr. L. J. Briggs, of the Bureau of Plant Industry. The determination of the moisture equivalent has been found to be convenient of execution, and the results from day to day are very concordant.

In Table III are given the moisture equivalent, the hygroscopic coefficient, the ratio of these to one another, and the content of organic matter in 36 samples. The soils were collected from 30 virgin prairie fields in Nebraska, 5 near each of the six towns indicated in the table. All are from fields classified by the United States Bureau of Soils either as Marshall silt loams or as Colby silt loam. In each field 10 borings were made to a depth of 6 feet and composite samples prepared of each foot section, thus securing 6 samples from each field. From these were prepared the samples used in this work, equal weights of the corresponding 5 field samples being combined. The details of the method of sampling are reported elsewhere (2, p. 204). In the same article (2, p. 215) are given the hygroscopic coefficients for the foot sections from each of all the fields. Each value in B of Table III represents the average of 10 determinations. The data on the organic matter reported in D of the table were calculated from the organic carbon reported in the same article (2, p. 228; organic matter =  $C \times 1.724$ ). The data on the moisture equivalents are the means of duplicate determinations.

The ratio (Table III-C) averages 2.38, varying from 2.14 to 2.73, a quite similar, although somewhat narrower, range than that found by Briggs and Shantz. In general, in each area it is highest in the surface foot as though influenced by the proportion of the organic matter.

TABLE III.—Moisture equivalent, hygroscopic coefficient, ratio of these two values, and organic-matter content of the foot sections from six different areas in Nebraska

## (A) MOISTURE EQUIVALENT

Depth.	Wauneta.	McCook.	Hol-drege.	Hastings.	Lincoln.	Weeping Water.	Average.
<i>Feet.</i>							
1.....	22.3	24.0	26.7	26.2	30.7	30.3	26.7
2.....	22.1	24.8	27.6	28.6	31.5	31.2	27.6
3.....	23.0	24.6	26.8	28.2	29.2	30.9	27.1
4.....	23.3	23.6	25.1	26.9	27.8	29.2	26.0
5.....	21.1	22.5	24.1	26.5	28.3	28.2	25.1
6.....	19.8	22.1	24.0	26.6	26.3	28.3	24.8
Average.....	21.9	23.6	25.7	27.2	29.3	29.7	26.2

## (B) HYGROSCOPIC COEFFICIENT

1.....	9.1	10.0	10.1	9.6	12.0	12.1	10.5
2.....	9.6	10.9	11.2	11.6	14.4	13.7	11.9
3.....	9.7	10.7	11.3	12.4	13.6	13.9	11.9
4.....	9.9	9.7	10.2	11.1	13.0	13.0	11.1
5.....	9.0	9.1	9.6	10.7	12.8	12.6	10.6
6.....	8.3	9.1	9.4	10.7	12.7	12.5	10.5
Average.....	9.3	9.9	10.3	11.0	13.1	13.0	11.1

## (C) RATIO OF MOISTURE EQUIVALENT TO HYGROSCOPIC COEFFICIENT

1.....	2.45	2.40	2.64	2.73	2.56	2.50	2.55
2.....	2.30	2.28	2.46	2.47	2.19	2.28	2.32
3.....	2.37	2.30	2.37	2.27	2.15	2.22	2.28
4.....	2.35	2.43	2.46	2.42	2.14	2.25	2.34
5.....	2.34	2.47	2.51	2.48	2.21	2.24	2.38
6.....	2.39	2.43	2.55	2.49	2.23	2.26	2.39
Average.....	2.37	2.38	2.50	2.48	2.25	2.29	2.38

## (D) PERCENTAGE OF ORGANIC MATTER

1.....	2.77	2.85	3.90	3.55	4.96	4.98	3.83
2.....	1.38	1.44	1.86	1.81	2.28	3.02	1.96
3.....	1.09	.97	1.01	.98	1.14	1.38	1.09
4.....	.79	.59	.66	.60	.60	.83	.68
5.....	.55	.48	.41	.41	.43	.45	.45
6.....	.45	.36	.36	.31	.40	.36	.37
Average.....	1.17	1.11	1.37	1.28	1.63	1.84	1.40

Table IV shows the values for the hygroscopic coefficients calculated from the moisture equivalents, using the Briggs-Shantz formula, and the departure from those directly determined. In all cases the values are more or less too low; using these there might appear to be as much as from 1.0 to 2.8 per cent of free water in the case of a subsoil which actually carried none.

TABLE IV.—*The hygroscopic coefficients calculated from the moisture equivalents and the departure of these from the values obtained by direct determination*

(A) CALCULATED HYGROSCOPIC COEFFICIENTS

Depth.	Wauneta.	McCook.	Holdrege.	Hastings.	Lincoln.	Weeping Water.	Average.
<i>Feet.</i>							
1.....	8.2	8.9	9.9	9.7	11.3	11.2	9.9
2.....	8.2	9.2	10.2	10.6	11.6	11.5	10.2
3.....	8.5	9.1	9.9	10.4	10.8	11.4	10.0
4.....	8.6	8.7	9.3	9.9	10.3	10.8	9.6
5.....	7.8	8.3	8.9	9.8	10.4	10.4	9.3
6.....	7.3	8.2	8.9	9.8	10.4	10.4	9.2
Average.....	8.1	8.7	9.5	10.0	10.8	11.0	9.7

(B) DEPARTURE FROM DIRECTLY DETERMINED VALUES

1.....	-0.9	-1.1	-0.2	-0.1	-0.7	-0.9	-0.6
2.....	-1.4	-1.7	-1.0	-1.0	-2.8	-2.2	-1.7
3.....	-1.2	-1.6	-1.4	-2.0	-2.8	-2.5	-1.9
4.....	-1.3	-1.0	-.9	-1.2	-2.7	-2.2	-1.5
5.....	-1.2	-.8	-.7	-.9	-2.4	-2.2	-1.3
6.....	-1.0	-.9	-.5	-.9	-2.3	-2.1	-1.3
Average.....	-1.2	-1.2	-.8	-1.0	-2.3	-2.0	-1.4

Table V gives similar data on another set of samples from the same 30 fields. These consisted of 1-inch sections from the surface foot (2, p. 206). In the case of these, however, each datum on hygroscopic coefficients as well as on moisture equivalents is the mean of only duplicate determinations. The ratio averages 2.75, compared with 2.71 found by Briggs and Shantz (Table I), and varies from 2.33 to 3.29, a range of 41 per cent, compared with 40 found by them with their 17 soils. Their samples also were probably surface soils rather than subsoils, such as predominate in Table III. In the inch sections, as in the foot sections, a decrease in the ratio is to be observed in passing from the surface to the subsoil. This may be attributed to the organic matter which appears to have a marked influence upon the moisture equivalent, although it shows little effect upon the hygroscopic coefficient (2, p. 217). Briggs and McLane (5, p. 18), found that organic matter had practically the same effect upon the moisture equivalent as an equal amount of clay.

TABLE V.—Moisture equivalent, hygroscopic coefficient, ratio of these two values, and the organic content of the inch sections of the surface foot

## (A) MOISTURE EQUIVALENT

Depth.	Wauneta.	McCook.	Holdrege.	Hastings.	Lincoln.	Weeping Water.	Average.
<i>Inches.</i>							
1.....	24.4	24.3	31.6	31.5	31.0	32.7	29.2
2.....	22.3	22.2	29.6	27.6	30.0	31.5	27.2
3.....	22.5	22.9	28.3	27.3	30.2	31.3	27.1
4.....	22.3	23.3	28.3	27.6	30.5	30.6	27.1
5.....	22.6	24.5	27.5	27.3	29.9	31.7	27.2
6.....	22.5	25.7	27.7	27.8	30.0	31.9	27.6
7.....	22.3	25.4	28.1	27.5	30.1	32.4	27.6
8.....	22.3	25.6	27.9	27.3	30.3	31.9	27.5
9.....	22.8	27.2	28.0	27.6	30.9	31.4	28.0
10.....	22.8	27.3	28.2	27.4	30.7	32.2	28.1
11.....	23.0	27.9	28.0	27.9	30.0	31.9	28.1
12.....	23.0	26.8	28.1	28.4	30.8	32.3	28.2
Average.....	22.7	25.2	28.4	27.9	30.4	31.8	27.7

## (B) HYGROSCOPIC COEFFICIENT

1.....	8.5	8.5	10.9	10.9	11.5	11.5	10.3
2.....	8.2	8.3	10.3	9.7	11.2	11.0	9.8
3.....	8.2	8.4	9.9	8.9	11.0	11.0	9.6
4.....	8.3	8.3	9.5	8.5	11.1	11.1	9.5
5.....	8.2	8.7	9.4	8.3	11.4	11.2	9.5
6.....	8.6	9.3	9.4	9.0	11.8	11.2	9.9
7.....	8.7	9.5	9.7	9.5	11.9	11.5	10.1
8.....	8.8	9.8	9.9	9.5	12.1	12.1	10.4
9.....	8.6	9.9	10.0	9.5	13.0	12.3	10.6
10.....	8.8	10.3	10.4	9.7	12.6	12.6	10.7
11.....	9.0	10.3	10.2	10.0	12.9	12.5	10.8
12.....	8.7	10.2	10.2	10.2	13.1	12.8	10.9
Average.....	8.6	9.3	10.0	9.5	12.0	11.7	10.2

## (C) RATIO OF MOISTURE EQUIVALENT TO HYGROSCOPIC COEFFICIENT

1.....	2.87	2.86	2.90	2.89	2.70	2.84	2.84
2.....	2.72	2.68	2.87	2.85	2.68	2.86	2.78
3.....	2.74	2.73	2.86	3.07	2.75	2.85	2.84
4.....	2.90	2.81	2.98	3.25	2.75	2.76	2.87
5.....	2.76	2.82	2.93	3.29	2.62	2.83	2.88
6.....	2.62	2.76	2.95	3.09	2.54	2.85	2.79
7.....	2.56	2.67	2.90	2.80	2.53	2.82	2.73
8.....	2.53	2.61	2.82	2.87	2.50	2.64	2.66
9.....	2.65	2.75	2.80	2.91	2.38	2.56	2.68
10.....	2.59	2.65	2.71	2.83	2.44	2.56	2.63
11.....	2.56	2.71	2.74	2.79	2.33	2.55	2.62
12.....	2.64	2.63	2.75	2.78	2.35	2.52	2.61
Average.....	2.66	2.72	2.85	2.96	2.55	2.72	2.74

TABLE V.—*Moisture equivalent, hygroscopic coefficient, ratio of these two values, and the organic content of the inch sections of the surface foot—Continued.*

(D) PERCENTAGE OF ORGANIC MATTER

Depth.	Wauneta.	McCook.	Holdrege.	Hastings.	Lincoln.	Weeping Water.	Average.
<i>Inches.</i>							
1.....	4.91	4.17	7.93	7.79	8.10	7.79	6.78
2.....	3.64	3.35	6.03	5.46	6.29	6.39	5.19
3.....	3.19	3.27	4.95	4.45	5.70	5.60	4.53
4.....	2.88	3.14	4.22	3.93	5.37	5.29	4.14
5.....	2.55	2.84	3.74	3.50	4.89	4.87	3.73
6.....	2.52	2.66	3.46	3.20	4.72	4.56	3.52
7.....	2.26	2.48	3.05	3.00	4.31	4.08	3.21
8.....	2.19	2.31	2.97	2.88	4.12	4.03	3.08
9.....	2.12	2.17	2.79	2.74	3.98	3.95	2.96
10.....	1.97	2.00	2.67	2.64	3.59	3.76	2.77
11.....	1.77	1.84	2.58	2.62	3.40	3.69	2.65
12.....	1.77	1.72	2.50	2.50	3.26	3.60	2.56
Average.....	2.67	2.69	3.90	3.72	4.77	4.81	3.76

The samples reported in Table VI are partly from the loess of Nebraska and partly from the residual soils of that State. A few are from New Mexico, Arizona, and California. The data upon both the hygroscopic coefficient and the moisture equivalent are the means of 5 to 10 concordant determinations. Nine of the samples are from the surface 6 to 12 inches, and the seven others from the subsoil. The range in texture represented by them is much the same as that of the soils reported by Briggs and Shantz (Table I).

TABLE VI.—*Relation of the moisture equivalent to the hygroscopic coefficient in a series of soils showing a wide range in texture*

Soil No.	Description of soil.	Moisture equivalent.	Hygroscopic coefficient.	Ratio of moisture equivalent to hygroscopic coefficient.	Hygroscopic coefficient calculated from moisture equivalent. <sup>1</sup>	Departure of calculated from found hygroscopic coefficient.
1	Desert sand, Palm Springs, Cal. . .	1.6	0.9	1.77	0.6	-0.3
2	Sandy subsoil, Palm Springs, Cal. .	2.8	1.1	2.54	1.0	- .1
3	Desert sand, Orogrande, N. Mex. . .	3.0	1.7	1.76	1.1	- .6
4	Sandy surface, W. Nebraska. . . . .	7.9	3.3	2.39	2.9	- .4
5	Sandy subsoil (A), W. Nebraska. . .	7.2	3.4	2.12	2.6	- .8
6	Sandy subsoil (B), W. Nebraska. . .	7.5	3.4	2.21	2.8	- .6
7	Sandy loam subsoil, W. Nebraska. . .	13.5	5.6	2.41	5.0	- .6
8	Sandy loam surface, W. Nebraska. . .	16.8	7.1	2.37	6.2	- .9
9	Silt loam subsoil (A), W. Nebraska. .	19.7	7.6	2.59	7.3	- .3
10	Silt loam subsoil (B), W. Nebraska. .	21.2	8.2	2.59	7.8	- .4
11	Red loam surface, Cuervo, N. Mex. .	19.2	10.0	1.92	7.1	-2.0
12	Silt loam surface (A), W. Nebraska. .	22.5	10.1	2.23	8.3	-1.8
13	Silt loam surface, E. Nebraska. . . .	27.8	10.2	2.73	10.3	.1
14	Silt loam surface (B), W. Nebraska. .	24.1	10.5	2.30	8.0	-1.6
15	Adobe surface, McNeal, Ariz. . . . .	25.8	12.9	2.00	9.6	-3.3
16	Silt loam subsoil, E. Nebraska. . . . .	29.5	13.3	2.22	10.9	-2.4

<sup>1</sup> Using Briggs and Shantz formula  $M. E. = \text{hyg. coeff.} \times 2.71$ .

Excepting the two sands, 1 and 3, the average ratio of moisture equivalent to hygroscopic coefficient is 2.33, with a maximum of 2.73 and a minimum of 1.92. The lowest ratios are shown by the arid or semiarid soils, 1, 3, 11, and 15. The exceptional behavior of 11 and 15 is not to be attributed to error of determination, as, after finding these exceptional ratios, we made repeated determinations of both values. The ratios found for both subsoils and surface soils from Nebraska are quite similar to those reported in Table III, the average ratio, 2.38, being identical with that obtained for the 36 loess samples.

If the two sands, 1 and 3, in Table VI, be omitted, the variation of our ratios in Tables III, V, and VI are of much the same order as those of Briggs and Shantz, shown in Table I. Thus, the divergence in our conclusions as to the availability to plants of the portion of the soil moisture lying between the hygroscopic coefficient and the wilting coefficient is not to be explained by any differences in our respective methods of arriving at the value of the hygroscopic coefficient. Neither are there sufficient reasons to attribute it to the particular range of soils with which we have worked, for the data above show that our soils range as widely as those which they have employed.

Their data, as well as our own work, make it evident that in any accurate experiments to determine the relation of the nonavailable water of the soil to the hygroscopic coefficient it is not permissible to calculate the value of the latter from the moisture equivalent, unless a previous thorough investigation has been made to determine just what formula is applicable to the soil type in question. From the data of Lipman and Waynick it would appear that in the case of certain soils this indirect method would be scarcely allowable for even the crudest studies on soil moisture. However, in the case of any extensive study, involving many soil types, the same general conclusions as to the relation of the nonavailable moisture to the hygroscopic coefficient are to be expected, no matter whether the latter value be directly determined or be calculated from the moisture equivalent by the Briggs-Shantz or by some more satisfactory formula.

#### COMPUTATION OF THE MOISTURE EQUIVALENT FROM THE MECHANICAL ANALYSIS

Table VII shows the concordance of the moisture equivalents directly determined with the values computed from the mechanical analyses in the cases of the loess samples reported in Table III, using the formula proposed by Briggs and Shantz:

Moisture equivalent = 0.02 sands + 0.22 silt + 1.05 clay, and also a modified form of this formula:

Moisture equivalent = 0.14 sands + 0.27 silt + 0.53 clay. In these formulas "sands" include particles ranging from 2 to 0.05 mm. in diameter. The separates referred to in the table as "coarser fractions" include the particles ranging from 2 to 0.10 mm. It will be seen that the

formula of Briggs and Shantz gives values too low for the coarsest textured members of the series and too high for the finest textured. In the modified formula the value assigned to the clay is lowered, that to the "sands" much increased, and that to the silt slightly raised. This formula gives results in close concordance with the directly determined values. The explanation of the need of altering the values is not far to seek. As has already been pointed out in connection with the computation of the hygroscopic coefficients from the mechanical analyses of the same samples (3, p. 406), the material coarser than silt is chiefly very fine sand, consisting mainly of particles but little larger than the upper limit for silt, while the so-called "clay" contains a very large proportion of silt particles with a diameter not much less than 0.005 mm.

Briggs and McLane (5, p. 21), in applying their generalized formula based upon the analysis of 104 soils, found that for the Marshall series it was necessary to give the clay a lower value and also to make allowance for the content of organic matter. As has been mentioned above, the samples in Table VII belong to the Marshall and Colby series.

Thus, it appears that if the mechanical analyses are to be used for the computation of moisture equivalents, it will be necessary, at least in the case of some widely differing soil types, to employ several different formulas.

TABLE VII.—*Concordance of the values for the moisture equivalent obtained by computation from the mechanical analysis with those directly determined*

WAUNETA									
Depth.	Coarser fract (2.0-0.1 mm.).	Very fine sand (0.1-0.05 mm.).	Silt (0.05- 0.005 mm.).	Clay (0.005- 0.000 mm.).	Moisture equivalent.				
					Determined.	Computed by formula.		Departure, using formula.	
						B. and S. <sup>1</sup>	Mod. <sup>2</sup>	B. and S.	Mod.
<i>Feet.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>					
1.....	4.8	48.7	41.2	5.4	22.3	15.9	21.5	-5.4	-0.8
2.....	2.4	47.8	43.3	6.6	22.1	17.5	22.2	-4.6	.1
3.....	2.0	46.8	43.8	7.5	23.0	18.5	22.6	-4.5	-.4
4.....	1.7	47.6	41.3	9.5	23.3	20.1	23.1	-3.2	-.2
5.....	1.5	50.0	43.6	4.9	21.1	15.8	21.6	-5.3	.5
6.....	1.3	54.9	39.8	4.2	19.8	14.3	20.8	-5.5	1.0
Average.	2.3	49.3	42.2	6.3	21.9	17.0	21.9	-4.9	0

M'COOK									
1.....	3.7	39.0	48.6	8.7	24.0	20.7	23.7	-3.3	-0.3
2.....	2.6	37.8	50.1	9.5	24.8	21.8	24.2	-3.0	-.6
3.....	1.3	36.4	53.9	8.4	24.6	21.4	24.3	-3.2	-.3
4.....	1.4	38.9	52.4	7.4	23.6	20.0	23.7	-3.6	.1
5.....	1.8	39.3	52.6	6.3	22.5	19.0	23.3	-3.5	.8
6.....	1.2	40.4	51.8	6.6	22.1	19.2	23.3	-2.9	1.2
Average.	2.0	38.6	51.6	7.8	23.6	20.4	23.7	-3.2	.1

<sup>1</sup> Moisture equivalent = 0.02 sands + 0.22 silt + 1.05 clay.  
<sup>2</sup> Moisture equivalent = 0.14 sands + 0.27 silt + 0.53 clay.

TABLE VII.—Concordance of the values for the moisture equivalent obtained by computation from the mechanical analysis with those directly determined—Continued.

HOLDREGE									
Depth.	Coarser fract (2.1—0.1 mm.).	Very fine sand (0.1—0.05 mm.).	Silt (0.05— 0.005 mm.).	Clay (0.005— 0.000 mm.).	Moisture equivalent.				
					Deter- mined.	Computed by formula.		Departure, using formula.	
						B. and S.	Mod.	B. and S.	Mod.
<i>Feet.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>					
1.....	2.8	25.9	64.6	6.7	26.7	21.8	25.0	-4.9	-1.7
2.....	1.3	24.6	62.9	11.6	27.6	26.1	26.5	-1.5	-1.2
3.....	.8	26.5	62.5	10.5	26.8	25.3	26.2	-1.5	-.6
4.....	.9	27.8	64.8	6.4	25.1	21.6	24.9	-3.5	-.2
5.....	2.5	31.7	60.0	5.8	24.1	20.0	24.1	-4.1	.0
6.....	2.4	31.1	60.7	5.8	24.0	20.1	24.2	-3.9	.2
Average.	1.8	27.9	62.6	7.7	25.7	22.5	25.1	-3.2	-.6

HASTINGS									
1.....	3.9	23.9	64.6	7.6	26.2	22.8	25.4	-3.4	-0.8
2.....	2.7	20.3	64.5	12.5	28.6	27.8	27.3	-.8	-1.5
3.....	2.3	22.2	61.9	13.6	28.2	28.4	27.4	.2	-.8
4.....	2.1	21.5	62.4	14.0	26.9	28.9	27.6	2.0	.7
5.....	2.4	20.9	66.7	10.0	26.5	25.6	26.6	-.9	.1
6.....	2.2	20.7	67.2	9.9	26.6	25.6	26.6	-1.0	0
Average.	2.6	21.6	64.5	11.3	27.2	26.5	26.8	-0.7	-0.4

LINCOLN									
1.....	3.8	13.5	68.0	14.8	30.7	30.8	28.6	0.1	-2.1
2.....	3.7	9.8	67.6	18.9	31.5	35.0	30.2	3.5	-1.3
3.....	3.4	9.3	68.0	19.3	29.2	35.5	30.4	6.3	1.2
4.....	3.2	9.6	68.1	18.9	27.8	35.1	30.2	7.3	2.4
5.....	3.7	9.9	69.4	17.0	28.3	33.4	29.7	5.1	1.4
6.....	3.9	9.5	70.2	16.5	28.3	33.0	29.6	4.7	1.5
Average.	3.6	10.3	68.5	17.6	29.3	33.8	29.8	4.5	.5

WEEPING WATER									
1.....	4.2	9.7	72.2	13.9	30.3	30.8	28.8	0.5	-1.5
2.....	2.8	8.2	69.5	19.6	31.2	36.1	30.7	4.9	-.5
3.....	1.0	13.8	66.7	18.6	30.9	34.5	29.9	3.6	-1.0
4.....	.6	14.9	67.0	17.6	29.2	33.5	29.6	4.3	.4
5.....	.5	14.7	67.9	17.0	28.2	33.1	29.5	4.9	1.3
6.....	.5	15.0	67.5	17.1	28.3	33.1	29.5	4.8	1.2
Average.	1.7	12.7	68.5	17.3	29.7	33.5	29.7	3.8	0
Average of all..	2.3	26.7	59.7	11.3	26.2	25.6	26.2	-0.6	0



## SUMMARY

The hygroscopic coefficient may in most cases be calculated from the moisture equivalent with sufficient accuracy to permit its use in soil-moisture studies. For certain types of soil, however, the ratio departs so widely from that assigned by Briggs and Shantz that the indiscriminate use of the latter value does not seem permissible. Before employing this indirect method for the determination of the hygroscopic coefficient in connection with soil-moisture studies the ratio should be experimentally established for each of the particular types of soil involved.

The effect of considerable quantities of organic matter is, in general, to give the ratio of the moisture equivalent to the hygroscopic coefficient a higher value.

In the case of any extensive study of soil moisture involving many soil types the same general conclusions as to the relation of the non-available moisture to the hygroscopic coefficient are to be expected no matter whether the latter value be directly determined or be calculated from the moisture equivalent by the Briggs-Shantz formula.

For the calculation of the moisture equivalent from the mechanical analysis no general formula appears universally applicable, the formula needing modification according to the soil type to which it is to be applied.

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# THERSILOCHUS CONOTRACHELI, A PARASITE OF THE PLUM CURCULIO

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## INTRODUCTION

During the seasons of 1914 and 1915 the ichneumonid *Thersilochus conotracheli* Riley (Pl. CIX) has been by far the most abundant and effective parasite of the plum curculio at North East, Pa. In 1914 a very large percentage of the fruit on a few plum trees (*Prunus* spp.) that stand on the premises of the Bureau of Entomology laboratory at that place was infested by the curculio. In the spring the adults of the parasite were abundant on these trees and parasitized a large percentage of the curculio larvæ in fruit that was still on the trees when the parasites became active.

Under date of June 13, 1914, the writer's notes contain the following:

Very few of the larvæ of this species have been found in host larvæ more than one-eighth inch long, although many of larger size than this have been examined. This indicates that the parasite does not begin oviposition until some time after the curculio has begun its attack on the fruit, and therefore does not exercise any control over the early curculio larvæ.

The season of 1915 found the curculio much reduced in numbers. The cold, wet season, however, retarded the emergence of the parasites to such an extent that they attacked only the latest of the larvæ, practically all of these being parasitized.

## HISTORICAL REVIEW

The first mention of *Thersilochus conotracheli* in literature appeared in 1871, when Riley (1) <sup>1</sup> published his original description of the species, referring it to the genus *Porizon*, and recorded it as a parasite of the plum curculio (*Conotrachelus nenuphar* Herbst) in New Jersey. Riley (2, p. 18) again referred to it in a paper written in German and published in St. Louis. In 1880 Gott (3, p. 57) reported that in his work with the plum curculio in Canada he had not found this parasite. Riley and Howard (4, p. 63-64) in 1889 referred the species to the genus *Thersilochus*, gave a brief life history, and recorded the species as nearly as

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<sup>1</sup> Reference is made by number to "Literature cited," p. 855.

abundant in some sections as *Sigalphus curculionis* Fitch. Thereafter until 1906 there were published apparently only two references to the species; one by Riley and Howard (5), which is a repetition of the original breeding record, and one by Harrington (6, p. 67), in which the insect is merely mentioned as a parasite of the plum curculio. In 1906 Johnson and Girault (7, p. 6) mentioned this insect in the account of their work on the plum curculio in New York, and accorded it small importance in the control of its host. Quaintance and Jenne (8, p. 147-148) in 1912 gave a résumé of previously published accounts, together with data on the abundance and emergence of adults in spring in New York and Pennsylvania. As showing the distribution of the species these authors list the following States: New York, New Jersey, Connecticut, Illinois, Missouri, and Kansas.

#### HOSTS AND DISTRIBUTION

So far as is known the plum curculio is the only insect attacked by this species, published records showing it to have been reared from this host in Connecticut, New York, New Jersey, Pennsylvania, Illinois, Missouri, and Kansas. In addition to these States the writer has had material from Michigan.

#### LIFE HISTORY OF THE SPECIES

##### GENERATIONS

This species is single-brooded, the life cycle of one generation embracing the whole year. The adult stage is reached in the fall, but the perfect insect does not leave its cocoon until the following spring.

##### EMERGENCE OF ADULTS

The adult *T. conotracheli* emerges from its cocoon from late May to the middle of June and very shortly begins the search for hosts.

##### RELATIVE ABUNDANCE AND TIME OF EMERGENCE OF SEXES

The males begin to appear a few days ahead of the females, and the latter continue to emerge long after the last male. A lot of cocoons collected by the late A. G. Hammar at Douglas, Mich., in the spring of 1911 and reared by the writer at Vienna, Va., produced adults as indicated in Table I. This table shows the date and period of emergence, the comparative dates of emergence of males and females, and the proportion of the sexes.

TABLE I.—Emergence of adults of *Thersilochus conotracheli* from cocoons collected at Douglas, Mich., and reared at Vienna, Va., in 1911

No.	Date emerged.	Males.	Females.
1.....	May 22	2	.....
2.....	23	1	.....
4.....	25	14	.....
6.....	27	11	1
7.....	28	1	2
8.....	29	1	2
9.....	30	2	2
10.....	31	2	8
11.....	June 1	3	6
12.....	2	1	3
13.....	3	.....	2
15.....	5	.....	11
16.....	6	.....	5
18.....	8	.....	3
19.....	9	.....	3
22.....	12	.....	3
24.....	14	.....	2
27.....	17	.....	1
28.....	18	.....	1
29.....	19	.....	1
30.....	20	.....	1
32.....	22	.....	1
33.....	23	.....	1
35.....	25	.....	1
37.....	27	.....	1
Total.....		38	61
Average date of emergence.....	May 27		June 7
Percentage of sexes.....		38.39	61.61

LONGEVITY OF THE ADULT

Some data on the longevity of the adult were obtained in 1911 from specimens reared at Vienna, Va., from the cocoons collected by Mr. Hammar at Douglas, Mich. Only females were used. Ten of these were divided into three lots, as follows: Four were placed in vials without food or water, three with water, and three with dilute sugar sirup. Of those without food or water one lived 4 days, two lived 3 days, and one lived 1 day. One of those provided with water lived 7 days, one 6 days, and one 4 days. Those fed with sirup lived 10 days, 13 days, and 15 days. The confinement in the vials undoubtedly shortened the life, even of the sirup-fed specimens, but the figures show the necessity for both food and water; for those given clear water lived, on the average, more than twice as long as those without food or water, and those provided with sirup lived more than four and one-half times as long.

STAGE OF HOST ATTACKED

Parasitization takes place while the curculio larva is still very small, probably from the time it hatches until in its burrowing into the fruit it gets beyond the reach of the ovipositor of the parasite.

## EFFECT OF PARASITISM ON HOST

Beyond possibly a temporary inactive condition induced by the sting, the oviposition by the parasite does not seem to injure the host. But shortly after the newly hatched larva begins to feed, the character of the body contents shows a considerable change. The adipose tissue loses its flocculent character and becomes a more homogeneous, more fluid mass, diluted by the blood. This same change in the host was noted by Timberlake (9) in his studies of *Limnerium validum* Cresson.

Parasitized larvæ at the time they leave the fruit are, as a rule, much smaller than healthy larvæ, although parasites have been found in hosts of nearly normal size. Whether this smaller size is due to the failure of the host to grow normally or to its failure to pass through all of its stages is a question which has not been determined.

## OVIPOSITION

In oviposition the female parasite, having found a curculio oviposition scar, raises her abdomen, at the same time releasing the ovipositor from its sheath and, directing it forward between her legs, thrusts it into the tunnel made by the curculio larva. If she can reach the larva, she pierces its skin and deposits within it a single egg. The act of oviposition is very brief, the longest observed having required about two minutes.

The female *T. conotracheli* has been observed repeatedly in the cages to attempt oviposition or rather to probe for possible hosts in abrasions of any sort in the skin of the plums provided. This apparently indicates that she can not recognize infallibly the typical scar made by the curculio in oviposition.

## INCUBATION PERIOD

No exact data on the incubation period are available, but that it is very short is indicated by the fact that in the many young larvæ examined in the course of the observations only one egg was found within a host, although they are very easily discovered. At the same time that the above-mentioned egg was discovered, another curculio larva, which had not traversed more than half an inch within the fruit, was found to contain a very young parasite larva. This also would indicate a short incubation period. A curculio larva exposed in a cage for one day, July 1-2, 1915, to the attack of *T. conotracheli* and dissected on July 7 was found to contain a very young larva of the parasite. This would indicate a maximum incubation period of six days, although it may have been even shorter than that.

## THE LARVA

POSITION IN RELATION TO HOST.—Throughout most of the larval life this species lives as an internal parasite, the larval parasite lying free within the body cavity of its host. When nearly full grown, however, it leaves the host and becomes temporarily an external feeder, draining from without the last trace of fluid from the body of its victim.

FEEDING PERIOD.—Because of the impossibility of following an individual parasite throughout its development, the determination of the exact duration of the various larval instars is very difficult and must be based on the average of many individuals. During this period of its life the larva molts four times. Larvæ of the first instar are to be found within curculio larvæ even as long as three days after the latter have finished feeding and entered the ground. In fact, it seems to be the rule that the first larval molt of the parasite takes place after the host has constructed its pupal cell. Apparently, however, this is not invariably true, since larvæ as old as the third instar have been removed from their hosts within three days of the time the latter entered the ground. Thereafter the development of the parasite is very rapid, for within 10 days it passes through its second, third, and fourth instars, and in some cases has left the body of its host and has begun the construction of its cocoon.

## DESTRUCTION OF SUPERNUMERARY LARVÆ.

Repeatedly in the dissection of the parasitized curculio larvæ more than one, sometimes several, first-instar larvæ of *T. conotracheli* have been found in a single host. Invariably, however, only one of these was in a healthy condition. The others were mostly dead and more or less inclosed in a mass of cells in the manner described by Timberlake (9, p. 75-76), and shown by him to be amebocytosis. In one case a still living but unhealthy larva partially inclosed by amebocytes was found in a curculio larva that also contained one healthy larva and two dead and completely invested larvæ. In no case, however, have all of the parasites been dead. Apparently the death of the parasite larvæ is not due to any protective adaptation on the part of the host, as suggested by Timberlake (9), for parasites in strange host species; for, as stated above, in no case were all larvæ killed, and in no case where but one egg was deposited within a host was the parasitism unsuccessful. The only source, therefore, of the destructive agency, whatever its nature, must lie within the surviving parasite larva. No explanation as to the nature of this agency is possible at this time.

## THE COCOON

The cocoon of *T. conotracheli* (fig. 1) is about 5 mm. long by about half as thick and oval in shape. It is constructed of tough reddish-brown silk.



FIG. 1.—*Thersilochus conotracheli*: Cocoon. Much enlarged.

## PUPATION AND TRANSFORMATION

About four or five days after the construction of the cocoon pupation takes place. When the transformation to the adult condition takes place was not determined by the present writer, but according to Quaintance and Jenne (8, p. 147-148) Mr. Fred Johnson found adults in cocoons at Youngstown, N. Y., in 1908, as early as August 24. As indicated above, the species hibernates in this condition within the cocoon.

## DESCRIPTION OF THE IMMATURE STAGES

## THE EGG

The egg (fig. 2) is oblong oval in shape, somewhat larger at the cephalic end, about 0.33 mm. in length by a little more than a fourth as wide, and slightly curved. A magnification of 215 diameters showed no sculpture of the chorion.



FIG. 2.—*Thersilochus conotracheli*: Egg. Highly magnified.

## THE LARVA

FIRST INSTAR.—The newly hatched larva (fig. 3) resembles in general appearance that of *Limmerium validum* as figured by Timberlake (9, p. 84).

The body consists of 13 segments, including the head and the long taillike caudal segment. The head is somewhat more than half as long as the rest of the body exclusive of the tail, which is somewhat longer

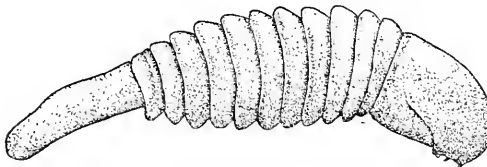


FIG. 3.—*Thersilochus conotracheli*: Larva of first instar. Highly magnified.

than the head. The head is bent slightly downward from the general axis of the body. It is heavily chitinized and pale brown in color, considerably longer than wide, and strongly curved above so that the

mouth opening is on the underside. The mouth parts consist of the heavily chitinized, acute, curved mandibles and the very delicate labrum, maxillæ, and labium. The exact form of the last three appendages is very difficult to ascertain with exactness, but they seem to be arranged about as in figure 4. The mandibles are very distinct even well within the head cavity, although their place of attachment is not clear. Ap-



parently they are attached very close together at a point above the labium. During its first instar the larva undergoes a comparatively enormous increase in size, becoming ultimately 2 mm. in length and much distended, only the head and taillike appendage retaining their original dimensions. The approximate appearance of the parasite at this period of its development is shown in figure 5.

SECOND INSTAR.—With its first molt the larva acquires an entirely different appearance. The first larval skin splits longitudinally just back of the head and the forward part of the body is drawn out. Then the skin is pushed off from the caudal end of the body. The head shield remains intact. The larva that emerges lacks the taillike appendage and the prominent, heavily chitinized head. Its mouth parts are apparently entirely soft, and the most careful preparation and mounting of specimens has failed to disclose any mandibles. The mouth has the appearance of a dimplelike depression without armature. At full growth this instar (fig. 6) is about 3 mm. long. The head measures 0.31 mm. broad.



FIG. 5.—*Thersilochus conotracheli*: Full grown larva of the first instar. Highly magnified.

broad. This measurement constitutes the only infallible distinction between the two stages. At full growth the third instar is about 3.50 mm. long.

FOURTH INSTAR.—With the assumption of the fourth instar the larva acquires the typical ichneumonoid larval characteristics. It is now in the form of a curved spindle, thick in the middle and tapering toward each end. It is about 4 mm. long with the head nearly a half millimeter broad, and with fairly distinct mouth parts. With high magnification the labrum, mandibles, maxillæ, and labium can be distinguished as well as the maxillary and labial palpi. The palpi appear merely as low rounded elevations on the surface of the maxillæ and labium. The mandibles are cone-shaped, and somewhat drawn out to

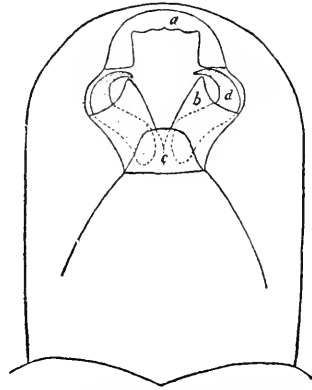


FIG. 4.—*Thersilochus conotracheli*: Ventral surface of head of first larval instar: a, Labrum; b, maxilla; c, labium; d, mandible. Highly magnified.

THIRD INSTAR.—The third instar is very like the second, except that it is larger, slightly stouter, and the head is 0.38 mm.



FIG. 6.—*Thersilochus conotracheli*: Larva of second instar. Greatly enlarged.

an acute, fairly strongly chitinized point. They are about 0.06 mm. long. The fourth molt takes place when the larva is about 4.75 mm. long.

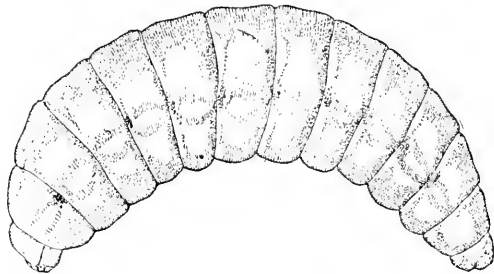


FIG. 7.—*Thersilochus conotracheli*: Full-grown larva, or fifth instar. Much enlarged.

The ridges supporting the mouth parts are strongly chitinized and can be faintly seen through the skin, especially the one extending from the base of the mandible around to the sides of the head. (See fig. 8.) Immediately after the molt this instar is about 5 mm. long, and at full growth, after it has left the body of its host and entirely consumed the fluid contents, it has reached a length of about 7 mm.

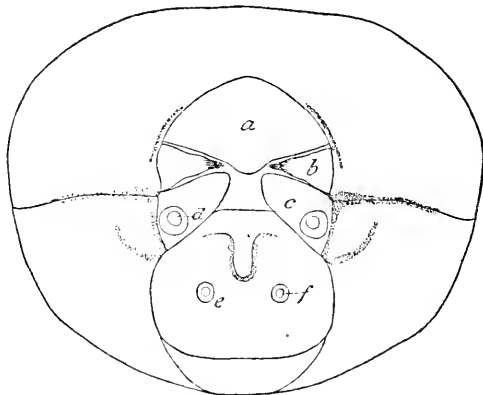


FIG. 8.—*Thersilochus conotracheli*: Face of full-grown larva: a, Labrum; b, mandible; c, maxilla; d, maxillary palpus; e, labium; f, labial palpus. Highly magnified.

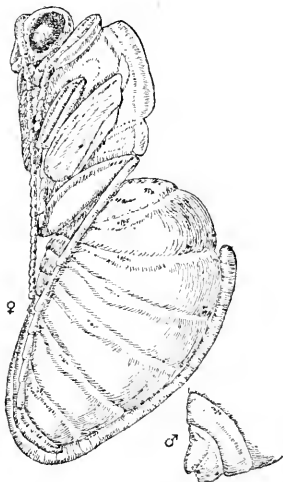


FIG. 9.—*Thersilochus conotracheli*: Pupa of female, and apex of abdomen of male pupa. Much enlarged.

#### THE PUPA

The pupa (fig. 9) is stout, about 4.5 mm. long, and in the female has the ovipositor curved up over the back and reaching about two-thirds of the way to the thorax. The abdomen is about two-thirds as deep as long and is much stouter than and nearly twice as long as the thorax. The thorax is blocky, with the rather small head situated near the ventral anterior margin. The antennæ reach to about the middle of the abdomen and the hind legs nearly to the apex. The abdomen of the male pupa is terminated by three lobes, one dorsal and two ventral, the latter representing the genital armature of the adult.

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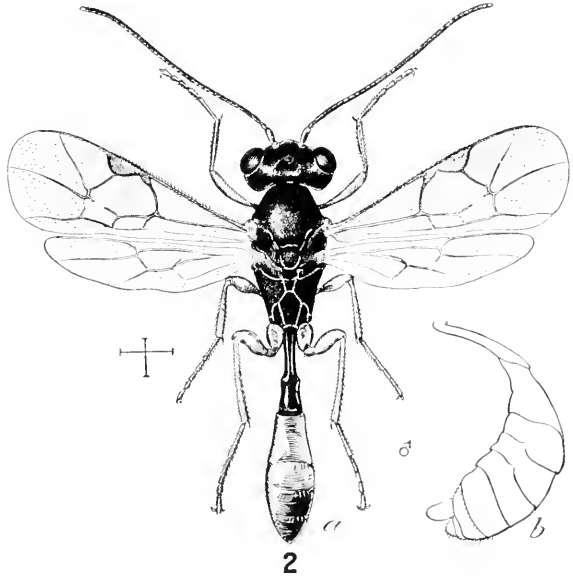
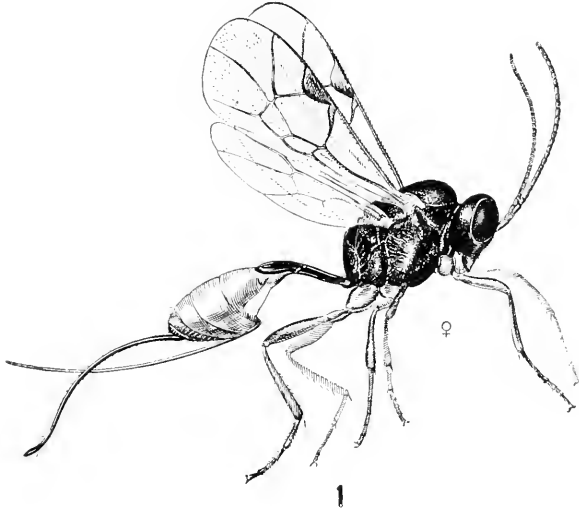
PLATE CIX

*Thersilochus conotracheli*:

Fig. 1.—Adult female. Greatly enlarged.

Fig. 2.—*a*, Adult male; *b*, side view of abdomen. Greatly enlarged.

(856)





# EFFECT ON PLANT GROWTH OF SODIUM SALTS IN THE SOIL

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## INTRODUCTION

In connection with an attempt to utilize for crop production certain salty land on the Truckee-Carson Field Station, at Fallon, Nev., it has been necessary to make numerous determinations as to the limit of the salt content of the soil tolerated by crop plants. These determinations have shown that this limit of tolerance is not a fixed and definite point, but is instead extremely variable. Not only is it influenced by many factors, such as kind of soil, kind of salt, and kind of plant, but the same crop plant shows marked differences in tolerance at different periods of its growth. These facts make the problem of dealing efficiently with the reclamation of alkali land a very complex one.<sup>1</sup>

In the present instance the more abundant and deleterious salts are those of sodium. These sodium salts occur as carbonates, bicarbonates, chlorids, and sulphates, and the proportions of each in different parts of the field are extremely variable. This variability of the proportions in which these salts occur confused the results of the early attempts to determine the limits of tolerance for the different crops. In order to establish a basis from which to proceed with the work, a series of pot cultures was carried on in which the soils were artificially impregnated with solutions of the different salts. These experiments have served to show the limit of tolerance to each of the four salts of one crop, wheat, in the seedling stage. They have also brought out a point which has not generally been taken into account in similar experiments—that the limit of tolerance of plants is dependent not upon the quantity of salt added to the soil but upon the quantity which exists in the soil solution and which is recoverable from the soil by water digestion.

It appears that the discrepancy between the amount of salt added to a soil and the amount which can be later recovered from it is sometimes very great. Different soils show different results in this respect; and some of the salts, particularly the carbonates and sulphates of sodium, are absorbed by the soil to a greater extent than sodium chlorid. Thus, if the limit of tolerance of a plant is given in terms of the quantity of salt which must be added to a soil to inhibit growth, this limit will be found

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<sup>1</sup> For literature germane to this subject see Harris, F. S. Effect of alkali salts in soils on the germination and growth of crops. *In Jour. Agr. Research*, v. 5, no. 1, p. 52-53. 1915.

to differ from one given in terms of the salt recoverable from the same soil. In actual field practice salt lands must be classified in terms of the amount of salt recoverable from them and not in terms of the amount which has been added to them, which is not ascertainable.

#### PLAN OF THE EXPERIMENTS

The general plan of all of the experiments was as follows: Ordinary drinking glasses were filled with 300 gm. of air-dry soil. The salts were added from a stock solution of known strength and ranged in amount from nothing up to concentrations sufficiently strong to prevent plant growth entirely. Distilled water was added to each glass to moisten the soil thoroughly. Seven seeds of wheat (*Triticum* spp.) were planted in each glass, and after germination the number of plants was reduced to five if more than that number came up. Bluestem wheat was used in 1913 and 1914, and Marquis wheat in 1915. To prevent loss of moisture, the glasses were covered with glass plates until the plants emerged. After the emergence of the plants, the pots were weighed daily, and by the addition of distilled water the moisture content was brought back to the original condition. In 1913 and 1914 the experiments were conducted in triplicate, while in 1915 they were in duplicate.

The wheat was allowed to grow from 15 to 18 days, when the plants were cut at the surface of the ground and weighed immediately in a closed tube. After cutting the plants the soils from each series of glasses were mixed, dried, and analyzed for water-soluble salts.

#### CONVERSION OF CARBONATES

In the experiments where sodium carbonate was added to the soil, the analysis of the soil after the wheat had been cut brought out the fact that a portion of the sodium carbonate that had been added was not recoverable.

When only a small quantity of sodium carbonate was added, none could be recovered at the end of the experiment, but the quantity of sodium bicarbonate was greater than in the untreated soil. With the addition of larger quantities of sodium carbonate both salts were recovered at the end of the experiment, but their sum was always less than the quantity added at the beginning.

It is apparent that a portion of the sodium carbonate added to the soil was converted into sodium bicarbonate. In order to determine what proportion of the original quantity of sodium carbonate could be accounted for at the end of the experiment, it was necessary to add together the quantity of sodium carbonate recovered as such and the quantity represented in the form of sodium bicarbonate.

The conversion of sodium carbonate to sodium bicarbonate results in an increase in weight of the salt at the ratio of 44 to 70—that is, the



weight of a quantity of sodium carbonate is 63 per cent of the weight of the sodium bicarbonate that could be formed from it.

In the following tables and discussions the sum of the sodium carbonate and 63 per cent of the sodium bicarbonate found in the soil solution have been designated as "carbonate salts."

EFFECT OF SODIUM CARBONATE ON WHEAT SEEDLINGS

EXPERIMENT 1.—The soil used in this experiment was obtained on the farm of the Truckee-Carson Field Station. It would be classed as a fairly productive sandy loam. It was analyzed for alkali salts and found to contain but a small quantity. The samples were made up in triplicate and sodium carbonate in solution was added to each set in the following percentages to the dry weight of the soil: Series 1, no treatment; series 2, 0.05; 3, 0.10; 4, 0.15; 5, 0.20; 6, 0.25; 7, 0.30; 8, 0.35; 9, 0.40; 10, 0.45; 11, 0.50; 12, 0.60.

Wheat was planted on November 1, 1913, and cut and weighed on December 11. Because of the lateness of the season, the growth had been very slow. After the wheat seedlings were removed, the soil from each set of pots was composited for analysis. The analysis was made of the solution secured by thorough digestion with an excess of water. The condensed results of this experiment are given in Table I.

TABLE I.—Results of experiment 1 (1913), giving the quantity of sodium carbonate added to the soil, the quantity finally recovered as carbonates, the number of plants, and the combined weight produced in each case

Series No.	Sodium carbonate.		Sodium bicarbonate recovered from soil. <sup>1</sup>	Total carbonate salts recovered.	Number of plants.	Green weight of plants.				Decrease in yield from check pot.
	Added to soil.	Recovered from soil.				Pot 1.	Pot 2.	Pot 3.	Total.	
	Per ct.	Per ct.	Per ct.	Per ct.		Gm.	Gm.	Gm.	Gm.	Per ct.
1.....	0	0	0	0	15	0.973	1.009	0.937	2.919	.....
2.....	.05	0	.02	.013	15	.978	.950	.974	2.902	0
3.....	.10	Trace.	.04	.025	15	.697	.865	.847	2.409	17
4.....	.15	.02	.04	.045	13	.619	.061	.116	.796	73
5.....	.20	.03	.05	.062	10	.220	.055	.044	.319	89
6.....	.25	.07	.05	.102	5	.008	.012	.020	.040	98.7
7.....	.30	.11	.02	.123	1	0	.008	0	.008	99.7
8.....	.35	.....	.....	.....	0	0	0	0	0	100

<sup>1</sup> In excess of sodium bicarbonate present at beginning of experiment.

This experiment showed that where more than 0.30 per cent of sodium carbonate was added to the soil no plant growth was obtained; therefore the analyses of the higher percentages have not been included in Table I. The addition of 0.15 per cent of the salt reduced the germination of the seed, so that the full number of plants was not obtained and the total green weight produced was 73 per cent below that of the check series.

Beyond this critical point the reduction of germination and growth was rapid and consistent.

The experiment also showed that the quantity of sodium carbonate recoverable at the end of the experiment was much less than had been added to the soil at the beginning. The quantity of sodium bicarbonate had been increased in every case, but the total carbonate salts recovered was much less than had been added.

The apparent loss of the sodium carbonate added to the soil is shown in the difference between the figures in columns 2 and 5 of the table. The data of Table I are shown graphically in figure 1.

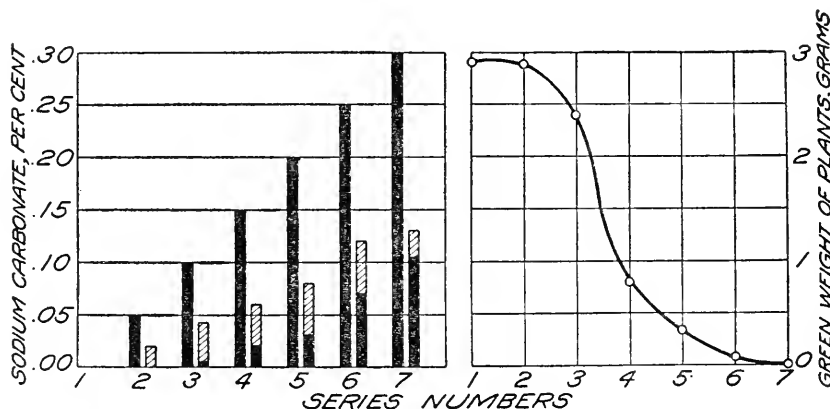


FIG. 1.—Diagram of the percentage of sodium carbonate added to the soil in experiment 1 (1913), with the percentage of carbonate and bicarbonate recovered and the total green weight of wheat obtained. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

EXPERIMENT 2.—The experiment was in most respects a repetition of experiment 1. The soil used was also taken from the same farm and was of the same physical character, but had been made more productive by the use of farm manure on the field from which it was taken. A preliminary analysis of this soil gave the following results: Sodium carbonate, 0; sodium bicarbonate, 0.091 per cent; sodium chlorid, 0.006 per cent; sodium sulphate, 0.

A triplicate series of sample pots were made up as before and sodium carbonate in solution was added as shown in Table II.

The wheat was planted in the pots on September 22, 1914, and cut and weighed on October 5. It was noted that with the successive increases in the percentage of carbonate added the time required for germination was increased, the percentage of germination decreased, and the amount of growth, both of leaves and roots, decreased. The results of this experiment are summarized in Table II.

**TABLE II.**—*Results of experiment 2 (1914), giving the quantity of sodium carbonate added to the soil, the quantity finally recovered as carbonates, the number of plants, and the combined weight produced in each case*

Series No.	Sodium carbonate.		Sodium bicarbonate recovered from soil. <sup>1</sup>	Total carbonate salts recovered.	Number of plants.	Green weight of plants.				Decrease in yield from check pot.
	Added to soil.	Recovered from soil.				Pot 1.	Pot 2.	Pot 3.	Total.	
	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>		<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Per ct.</i>
1.....	0	0	0	0	15	1.013	0.951	1.102	3.066	0
2.....	.05	0	0	0	15	1.025	.966	.778	2.769	10
3.....	.10	0	.03	.019	12	.697	.776	.554	2.027	33
4.....	.15	.02	.03	.039	11	.551	.680	.450	1.681	45
5.....	.20	.03	.03	.049	6	.450	.127	.327	.904	71
6.....	.25	.04	.04	.065	6	.064	.115	.281	.460	85
7.....	.30	.06	.04	.085	3	.028	0	.145	.173	94
8.....	.35	.07	.05	.102	3	.044	0	.273	.317	90
9.....	.40	.12	.03	.139	1	.020	0	.059	.079	97.5

<sup>1</sup> In excess of sodium bicarbonate present at beginning of experiment.

The results of experiment 2 are in close accord with those of No. 1, although the decrease in yield was not quite so rapid. The apparent loss of carbonates—that is, the difference between the amount added and that recovered—was slightly greater. It is noticeable that in both experiments the percentage of sodium bicarbonate recoverable did not increase materially with the percentage of carbonate added, while there was a fairly consistent increase in the percentage of carbonate recovered. Furthermore, it will be observed that the decrease in yield follows the increase in total carbonates recovered more closely than the increase in carbonate added to the soil.

The results of experiment 2 are shown graphically in figure 2, in which the same arrangement of symbols is used as in figure 1.

**EXPERIMENT 3.**—This experiment was undertaken for the purpose of comparing the toxic effect of sodium carbonate on the growth of wheat seedlings in two very different types of soil. The first of these was a rich loam soil from an old alfalfa field on the Truckee-Carson Irrigation Project, and the second was beach sand obtained from Monterey, Cal. The experiment was conducted in the summer of 1915. Duplicate sets of pots were used in each case. After the salt had been added to the pots, the moisture content was kept at 12 per cent in the sand and 15 per cent in the loam. This arrangement had the disadvantage of making the concentration of the soil solution different in the two soils, but it was considered necessary because 15 per cent of moisture in the sand would have kept it too wet, and less than 15 per cent in the loam soil would not have been sufficient for the best growth of the plants.

The difference in the effect of the carbonate in the two soils was evident in a very few days. The time required for the wheat to emerge was approximately the same in both cases, but in the sand all germination was stopped by the addition of 0.20 per cent of sodium carbonate, whereas it required the addition of 0.50 per cent to the loam to have the same effect. The carbonate had a very detrimental effect on the physical condition of the loam soil, causing a stiff crust to form on the top of the pots, the crust becoming more noticeable with the increase of the percentage of carbonate. This made it difficult for the plants to break through. The detailed results of the experiment with loam and sand soils are given in Table III.

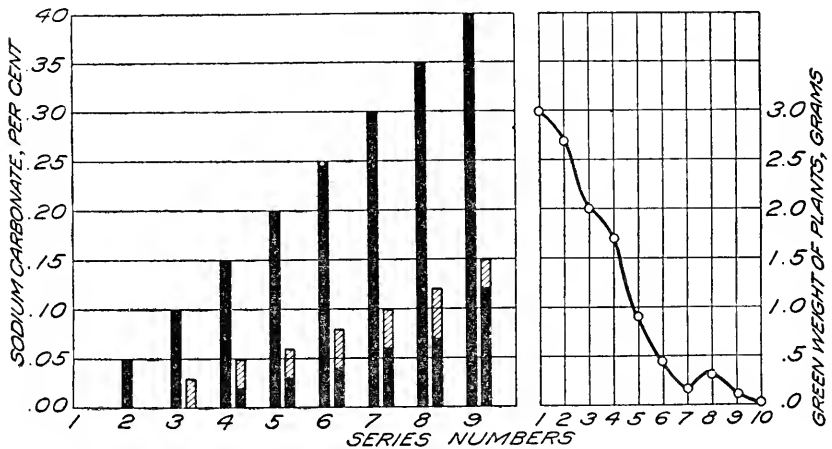


Fig. 2.—Diagram of the percentage of sodium carbonate added to the soil in experiment 2 (1914), with the percentage of carbonate and bicarbonate recovered and the green weight of wheat obtained. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

A comparison of the data on loam and Monterey sand (Table III) shows that the decrease in the yield of the plants was much more rapid in the sand than in the loam. The apparent loss of carbonates was much greater in the loam than in the sand. The loam soil also showed a steady increase in the amount of recoverable sodium bicarbonate, which was not the case with the sand.

A marked difference is to be noted in the green weight of the plants grown in the loam and in the sand. In the check pots the green yield from the sand series was only 62 per cent of the yield of the plants in the loam, although the average height of the plants in the two series was approximately the same.

The data presented in Table III are shown graphically in figures 3 and 4.

TABLE III.—Results of experiment 3 (1915), giving the effect of sodium carbonate in loam soil and in Monterey sand on the germination and growth of wheat

## LOAM SOIL

Series No.	Sodium carbonate.		Sodium bicarbonate recovered from soil. <sup>1</sup>	Total carbonate salts recovered.	Number of plants.	Green weight of plants.			Decrease in yield from check pot.
	Added to soil.	Recovered from soil.				Pot 1.	Pot 2.	Total.	
	Per ct.	Per ct.	Per cent.	Per cent.		Gm.	Gm.	Gm.	Per ct.
I.....	0	0	0	0	10	0.791	0.700	1.491	0
2.....	.05	0	.03	.019	10	.725	.782	1.507	0
3.....	.10	0	.05	.032	10	.535	.708	1.243	17
4.....	.15	0	.04	.025	10	.588	.629	1.217	19
5.....	.20	0	.05	.032	10	.666	.520	1.186	21
6.....	.25	0	.08	.050	9	.497	.420	.917	38.5
7.....	.30	0	.10	.063	8	.255	.538	.793	47
8.....	.35	0	.16	.101	5	0	.351	.351	76
9.....	.40	.008	.18	.122	4	.186	.077	.263	83
10.....	.50	.013	.21	.145	0	0	0	0	100
11.....	.60	.023	.22	.162	0	0	0	0	100

## MONTEREY SAND

I.....	0	0	0	0	10	.486	.445	.931	0
2.....	.05	.003	.066	.045	10	.176	.176	.352	62
3.....	.10	.013	.087	.068	6	.069	.030	.099	89
4.....	.15	.016	.119	.091	5	.027	.024	.051	96
5.....	.20	.049	.132	.132	0	0	0	0	100
6.....	.25	.080	.116	.153	0	0	0	0	100
7.....	.30	.096	.125	.175	0	0	0	0	100
8.....	.35	.140	.137	.226	0	0	0	0	100
9.....	.40	.244	.159	.344	0	0	0	0	100

<sup>1</sup> In excess of sodium bicarbonate in soil at beginning of experiment.

## THE EFFECT OF SODIUM BICARBONATE ON WHEAT SEEDLINGS

EXPERIMENT 4.—In order to determine the relative toxicity of the carbonate salts when added to a soil in the form of sodium bicarbonate, experiment 4 was undertaken in the summer of 1914. The technique of this experiment was the same as that of the experiments previously described, the series of pots being triplicated. The soil used was of the same type as that in experiment 2. The range of salts added to the soil was greater than in the first two experiments, including series numbered 8, 9, and 10, in which were added 0.80, 1, and 1.25 per cent of sodium bicarbonate. Since there was no germination or growth in these series, they have not been included in Table IV, which gives a summary of the results of the experiment.

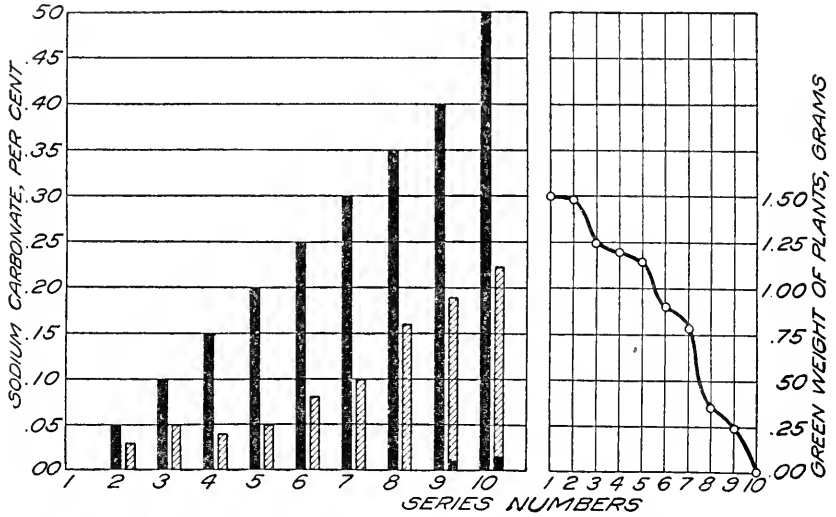


FIG. 3.—Diagram of the percentage of sodium carbonate added to the loam soil in experiment 3 (1915), with the percentage of carbonate and bicarbonate recovered, and green weight of wheat. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

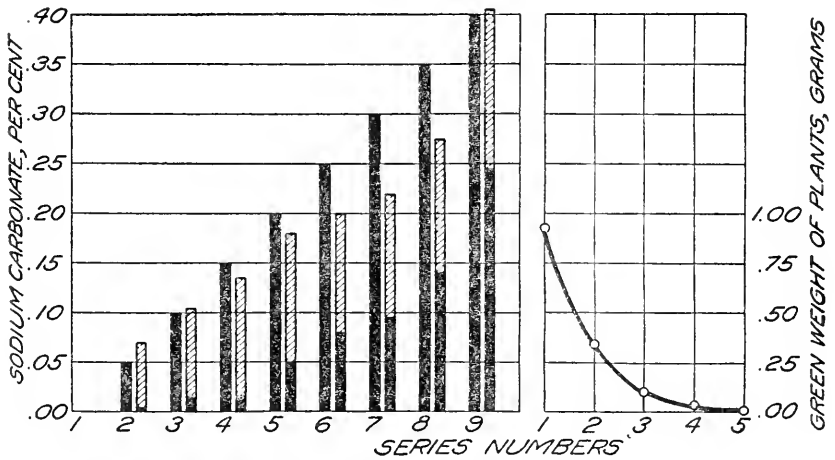


FIG. 4.—Diagram of the percentage of sodium carbonate added to Monterey sand in experiment 3 (1915), with the percentage of carbonate and bicarbonate recovered and the total green weight of wheat obtained. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

TABLE IV.—Results of experiment 4 (1914), giving the effect of sodium bicarbonate on the germination and growth of wheat

Series No.	Sodium bicarbonate.		Sodium carbonate recovered from soil.	Total carbonate salts recovered.	Number of plants.	Green weight of plants.				Decrease in yield from check pots.
	Added to soil.	Recovered from soil.				Pot 1.	Pot 2.	Pot 3.	Total.	
	Per ct.	Per ct.	Per ct.	Per ct.		Gm.	Gm.	Gm.	Gm.	Per ct.
1	0	0	0	0	15	0.996	0.917	1.032	2.945	0
2	.05	0	0	0	15	1.051	.790	.905	2.746	6.8
3	.10	.012	0	.008	15	.856	.841	.929	2.626	10.8
4	.20	.029	0	.018	15	.981	.873	.819	2.673	9.2
5	.30	.071	0	.045	15	.362	.460	.371	1.193	59.5
6	.40	.088	.021	.076	5	.065	.114	.070	.249	91.5
7	.60	.071	.074	.119	0	0	0	0	0	100

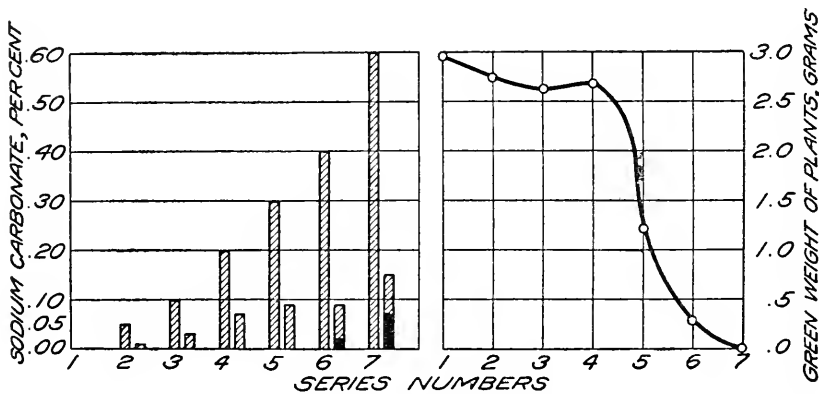


FIG. 5.—Diagram of the percentage of sodium bicarbonate added to the soil in experiment 4 (1914), with carbonate and bicarbonate recovered, together with the total green weight of wheat obtained. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

The first noticeable feature of this experiment is the discrepancy between the amount of bicarbonate added to the soil and the amount finally recovered. There was also evidence of a conversion of the bicarbonate to the carbonate form in the last two series. In this case, as in experiment 2, there was a decrease of growth in series 2, even though no carbonate salt was recoverable at the end of the experiment.

The data presented in Table IV is shown graphically in figure 5.

COMPARATIVE TOXICITY OF SODIUM CARBONATE AND SODIUM BICARBONATE

A comparison of experiments 2 and 4, in which the carbonate and bicarbonate salts were used, shows that these two salts have approxi-

mately the same toxic effect when the total of the carbonate salts recoverable is considered rather than the percentage of salts added to the soil. In other words, the toxicity of these salts in the soil is directly associable with the quantity of the basic radical in the salt recoverable. The close relationship between the results of these two experiments is shown in figure 6, in which the curves of decrease in growth are constructed on the same scale.

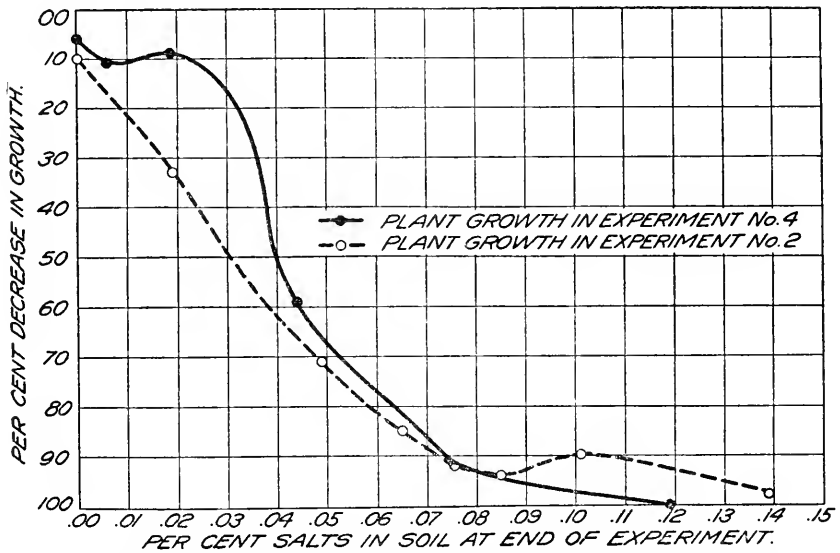


FIG. 6.—Diagram of the decrease in growth of wheat seedlings in experiments 2 and 4 as affected by the total carbonate salts recoverable from the soil. The solid black line on the left at each series number indicates the percentage of sodium carbonate added to the soil; the line at the right shows the percentage recovered at the end of the experiment. The solid portion of the line shows the carbonate and the hatched portion the bicarbonate. The curve at the right of the figure shows the relative growth of the plants in each series of pots.

#### EFFECT OF SODIUM CHLORID ON WHEAT SEEDLINGS

EXPERIMENT 5.—At the same time the carbonate and bicarbonate experiments in 1914 were in progress (experiments 2 and 4) a similar experiment with sodium chlorid was carried on with the same soil. The general plan and manipulation was the same as has been described above. The wheat was allowed to grow for 16 days. The original soil contained only 0.006 per cent of sodium chlorid.

A summary of the results obtained in this experiment is given in Table V. It is possible to compare these results directly with those obtained in the carbonate, bicarbonate, and sulphate experiments in 1914, as the soil used was the same in all cases.



TABLE V.—Results of experiment 5 (October, 1914), giving the effect of sodium chlorid in soil on the germination of wheat seedlings

Series No.	Sodium chlorid.		Number of plants.	Green weight of plants.				Decrease in yield from check pots.
	Added to soil.	Recovered from soil.		Pot 1.	Pot 2.	Pot 3.	Total.	
	Per ct.	Per ct.		Gm.	Gm.	Gm.	Gm.	
1			15	0.943	0.852	0.980	2.776	
2	0.05	0.04	15	.824	.818	.821	2.464	11.0
3	.10	.09	13	.724	.646	.642	2.013	27.5
4	.20	.16	13	.420	.430	.562	1.412	49.0
5	.30	.26	5	.115	.093	.178	.388	86.0
6	.40	.32	5	0	.029	.015	.044	98.4
7	.60	.52	0	0	0	0	0	100.0

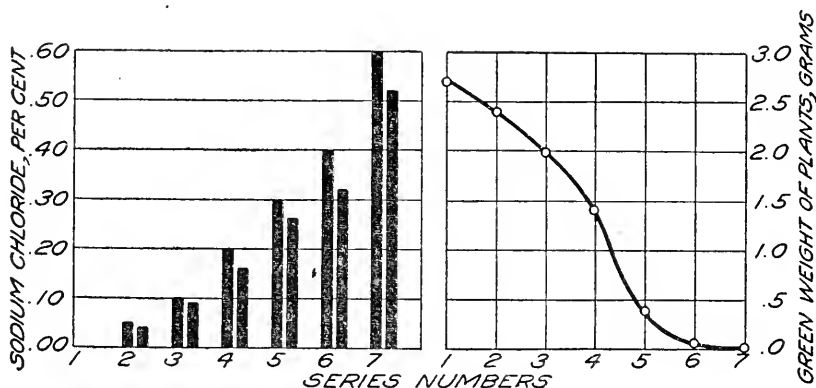


FIG. 7.—Diagram of the quantity of sodium chlorid added to the soil, with the quantity of chlorid recovered, and the total green weight of wheat obtained. Experiment 5. The left-hand column above the series number represents the percentage of sodium chlorid added and the right-hand column the percentage recovered from the soil. The curve at the right represents the weight (in grams) of the green wheat.

It is clear from the results given in Table V that the absorptive power of the soil for sodium chlorid is much less than for sodium carbonate. An average of 85 per cent of the chlorid was recovered, and a 50 per cent decrease in yield took place in the soil from which 0.16 per cent of the salt was recovered.

The results of Table V are shown graphically in figure 7.

#### EFFECT OF SODIUM SULPHATE ON WHEAT SEEDLINGS

EXPERIMENT 6.—The experiment with sodium sulphate was conducted in the same manner and at the same time and with the same lot of soil as experiments 2, 4, and 5. The moisture content of the soil was kept at 16 per cent and the wheat was allowed to grow for 16 days. The results are summarized in Table VI.

TABLE VI.—Results of experiment 6 (1914), giving the effect of sodium sulphate on wheat seedlings

Series No.	Sodium sulphate.		Number of plants.	Green weight of plants.				Decrease in yield from check pot.
	Added to soil.	Recovered from soil.		Pot 1.	Pot 2.	Pot 3.	Total.	
	Per cent.	Per cent.		Gm.	Gm.	Gm.	Gm.	
1.....	0	0	15	0.041	0.957	0.916	2.815	.....
2.....	.05	0	15	.810	.968	.795	2.574	8.5
3.....	.10	.07	15	.975	.687	.815	2.778	0.5
4.....	.20	.16	14	.728	.573	.936	2.238	20.5
5.....	.30	.19	14	.610	.597	.713	1.920	31.5
6.....	.40	.25	15	.685	.693	.592	1.971	30
7.....	.60	.35	15	.513	.360	.430	1.303	53.5
8.....	.80	.37	11	.091	.277	.346	.715	75
9.....	1.00	.45	10	.028	.163	.076	.268	96.5
10.....	1.25	.56	0	0	0	0	0	100

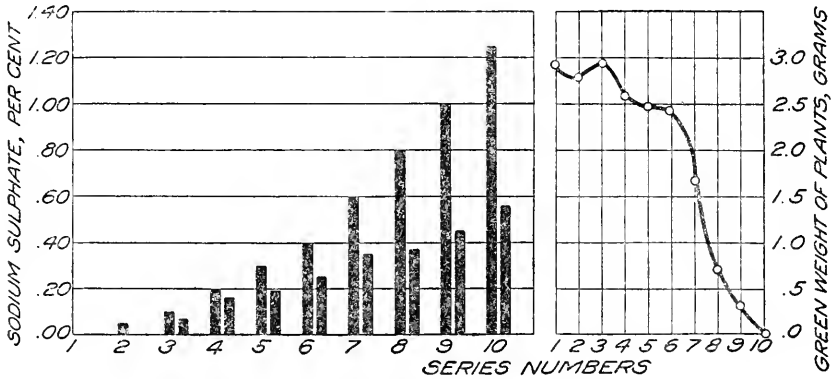


FIG. 8.—Diagram of the quantity of sodium sulphate added to the soil in experiment 6, the quantity recovered, and the total green weight of wheat obtained. The left-hand column above the series number represents the quantity of sodium sulphate added and the right-hand column the quantity recovered. The curve at the right represents the weight (in grams) of green plants from each series.

In the preceding experiments it was found that this same sandy loam soil absorbed an average of 77 per cent of the sodium carbonate, 85 per cent of the sodium bicarbonate, and 15 per cent of the sodium chlorid added. In this experiment with sodium sulphate it was found that the amount absorbed was 53 per cent of that added.

The toxicity of this salt was also considerably less than that of any of the other salts mentioned. In the case where 0.16 per cent of the sulphate was recovered, the yield was reduced only 20 per cent, while an equal amount of sodium chlorid reduced the yield 49 per cent. The percentage of germination was not affected in the case where 0.35 per cent of sodium sulphate was recovered, but it was noticed that the time required for germination was materially lengthened as the percentage of the sulphate increased.

Figure 8 shows graphically the results given in Table VI.

## COMPARATIVE TOXICITY OF THE SODIUM SALTS

In view of the fact that the carbonate and bicarbonate of sodium appear to be interchangeable in the soil, the comparisons of toxicity may be made between the total carbonate salts as previously defined and sodium chlorid and sodium sulphate. Assuming that a reduction of growth of approximately 50 per cent of the check is a critical point of toxicity at which comparisons can be made, it is found that this point is reached with 0.04 per cent of total carbonate salts, with 0.16 per cent of sodium chlorid, and 0.35 per cent of sodium sulphate, using the quantities of salt recoverable from the soil—that is, the carbonate salts are four times as toxic as the chlorids and eight times as toxic as the sulphates.

If the limit of tolerance for the wheat seedling is taken as the point of concentration when both growth and germination are prevented, this is found to be with the carbonate 0.13 per cent, with sodium chlorid 0.52 per cent, and with sodium sulphate 0.56 per cent. It is not clear why there is so little difference in these experiments between the limit of tolerance for sodium chlorid and sodium sulphate.

## SUMMARY

(1) In reclaiming a tract of salt land in Nevada laboratory experiments were carried on to determine the limits of tolerance of certain crop plants to the common salts of sodium.

(2) These laboratory experiments brought out the fact that only a part of the salt added to the soil in pot cultures could later be recovered from it by water digestion.

(3) This apparent loss of salt, which was probably due to absorption by the soil, was greater in the case of sodium carbonate and sodium sulphate than with sodium chlorid.

(4) Where sodium carbonate was added to a soil the absorption was greater in fine soil, rich in organic matter, than in sand.

(5) The limit of tolerance of crop plants to the salt in the soil is determined by the quantity of salt that can be recovered from the soil rather than by the quantity added to the soil.

(6) The carbonates and bicarbonates of sodium are mutually interchangeable in the soil and the toxicity of the soil solution appears to depend upon the quantity of the basic radical held in the soil regardless of the form of the acid radical.

(7) In the case of the soil from the field under consideration, the proportion of recoverable salt which would reduce by one-half the growth of wheat seedlings was for the carbonates 0.04 per cent of the dry weight of the soil, for the chlorids 0.16 per cent, and for the sulphates 0.35 per cent.

(8) The proportion of recoverable salt which prevented germination of wheat was for the carbonates 0.13 per cent, for the chlorids 0.52 per cent, and for the sulphates 0.56 per cent.

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# JOURNAL OF AGRICULTURAL RESEARCH

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# JOURNAL OF AGRICULTURAL RESEARCH

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No. 23

## OBSERVATIONS ON THE LIFE HISTORY OF THE ARMY CUTWORM, *CHORIZAGROTIS AUXILIARIS*<sup>1</sup>

By R. A. COOLEY,

*Entomologist, Montana Agricultural Experiment Station*

### INTRODUCTION

The army cutworm (*Chorizagrotis auxiliaris* Grote) occupies a prominent place among the pests of staple crops in the Northwest. Serious outbreaks have occurred at irregular intervals, and more or less damage is done nearly every year. Our knowledge of the life history of the species has been very incomplete, and further facts are obviously needed. The purpose of the present article is to present the results of studies made during several years and particularly some observations made in 1915 on the egg-laying habits of the species, together with their bearing on the question of the number of broods in the annual cycle.

### SCIENTIFIC NAME OF THE SPECIES

In previous years the writer has repeatedly reared to the adult stage larvæ which had been taken in destructive colonies of this insect. Among these he has found forms corresponding with descriptions of *Chorizagrotis auxiliaris*, *C. agrestis*, and *C. introferens*. Moths reared from much-rubbed parents caught in the fall of 1915, as recorded below, when determined were found to include at least two of these forms. Gillette<sup>2</sup> states that he had found these forms occurring together in colonies in Colorado. For these reasons and because *C. auxiliaris* has priority, this name has been used to designate this insect in the present paper.

### PREVIOUS EXPERIMENTAL EFFORTS

The attempts of the writer to obtain information regarding the life history of this species, and particularly regarding the egg-laying habits, date back for several years, and a brief summary of these efforts may be

<sup>1</sup> Since completing this manuscript and just as it is about to be offered for publication, the writer received a copy of Strickland's excellent paper, which covers somewhat the same ground. (Strickland, E. H., The army cutworm, *Euxoa* (*Chorizagrotis*) *auxiliaris* Grote. Canada Dept. Agr. Ent. Branch, Bul. 13, 1916.)

<sup>2</sup> Gillette, C. P. Some of the more important insects of 1903. *In* Colo. Agr. Exp. Sta. Bul. 94 (Tech. Ser. 6), p. 6. 1904.

of benefit to any who have occasion to search for the eggs of other Noctuidæ. On account of the irregular occurrence of the species, it has been quite impossible to depend upon getting a supply of living specimens for study when wanted, and a continuous effort in the search for the facts desired has been out of the question. In the nature of the case, instead of laying out and following a definite plan of study the successful pursuit of which would certainly lead to the results desired, it has been necessary to rely largely upon scattering observations made through several years as opportunity was afforded.

The first attempt to obtain the early stages was made in 1907, when, in the writer's first experience with an outbreak of the species, a large number of moths originating from larvæ which had been brought in during the fall, were reared during the winter in the insectary and were held in large Riley-type cages in order that they might have an opportunity to lay eggs. All of the moths died within a few days, and no eggs were laid. In the spring several hundred larvæ were brought in from grain fields and fed to maturity. The moths which emerged were left in the cages and given water and a variety of plants upon which to lay eggs. Again the moths died without laying eggs.

Several explanations for the failure suggested themselves. It was thought possible that the moths were not normal because of having been reared in confinement. A number of female moths were dissected for the purpose of examining the ovaries, and it was found that the ova were immature. The idea suggested itself that the failure to develop ova was the outworking of some little understood principle of periodicity in the occurrence of the species, and it was also thought that the absence of mature ova might be due simply to the lack of food after the emergence of the adults.

In the spring of 1910 there was a destructive colony of the larvæ 8 miles west of Bozeman, and plans were laid to recover a supply of the pupæ from the soil, allow them to emerge in confinement, and attempt to feed the adults on honey water to get them to grow ova. Our trip to the field to secure pupæ was not correctly timed, and the moths had already emerged. Accordingly, an attempt was then made to obtain moths by catching them at night near the same field. A special trap light was arranged, consisting of a wooden box 18 inches square and 12 inches high with an 8-inch circular hole in the top into which was inserted a large funnel-shaped tin with a 2-inch opening at the bottom. Above the funnel was placed an acetylene light from a stereopticon. This furnished a very strong light, and the night was favorable. The writer and an assistant went to the field, expecting to spend the night. They remained until between 12 and 1 o'clock, but only a few moths of any kind came, and none of *C. auxiliaris*. As no encouragement whatever was received, even though it was known that many thousands of



moths had emerged recently in the vicinity, the experiment was abandoned.

On numerous occasions attempts have been made to find moths in the field in the act of depositing eggs, either on crops or on vegetation on virgin sod. Much time has also been spent in searching for eggs on grasses, clover, alfalfa, stools of volunteer grain, and other cultivated plants. A few eggs of other noctuids have been found, but none of this species.

#### CAGE EXPERIMENTS

In April and May, 1915, there occurred in Montana a widespread and very severe outbreak of the army cutworm. Moving armies of the larvæ were reported daily, and many thousands of acres of fall wheat were eaten off during April and May, so that it was possible to obtain a supply of the living insects for study. Plans were made for a twofold effort in connection with the outbreak. It was decided to install several large cages outdoors and by any means possible to obtain adult moths of both sexes, place them in the cages, and attempt to feed and keep them alive until they should lay eggs, correlating the observations made in these cages with notes from the field.

Three large cages 24 inches square by 40 inches high were installed on the lawn near the insectary. These are of fine-mesh brass screening and are fastened to the earth by a broad baseboard which is inserted in the soil. A large door fills one side and in this door is a smaller one, sufficiently large to permit the insertion of the hand. On the 24th of April 28 larvæ were placed in cage 2 and 53 in cage 3. These worms had been collected from two fields at Willow Creek, Mont. They were fed regularly, but did not do well. It has been repeatedly found that it is difficult to rear a large proportion of these caterpillars when fed in a body in one cage. For this reason in rearing record specimens the writer has adopted the method of feeding the caterpillars individually in tin boxes and by so doing has brought nearly every individual to maturity. None of these caterpillars pupated in the large cages.

On June 10 about 300 pupæ which had been taken from the soil in a field at Willow Creek were brought to Bozeman and placed in cages 1 and 2. Not one of these emerged. When examined, they were dead and decayed. They had been placed individually in holes in the soil with the anterior end uppermost—a method which has been used with dry soil with much success in indoor rearing. It is possible that rains had closed the holes and injured the pupæ by puddling the earth around them. On the 20th of June, 13 moths which had been reared in the insectary were placed in cage 1, and on July 17 about 50 moths which were captured out of doofs at Willow Creek were divided between cages 1 and 2. It was hoped that some of these would grow ova and deposit eggs. Sponges saturated with honey water were placed in the cages daily and clover

blossoms were picked and put in fresh each day. A variety of plants in small pots were placed in the cages, and as some of these blossomed, it is probable that they would furnish more or less nectar on which the moths might feed. The general condition in these cages certainly more closely simulated complete liberty than could have been provided in the insectary.

The writer was at first much disappointed in the results obtained, for by repeated examinations of the vegetation which had been placed in the cages, he was unable to find any eggs. The moths lived on, however, and served a very valuable purpose in indicating that the normal life of the moth in the open might be much longer than had been thought. While the moths gradually died off during the summer, many were alive on August 16, and several were seen as late as September 21. A pair was seen in copulation on August 10. Since no eggs had been found in the cages, the fact that the moths lived on until so late also suggested that perhaps the period between the emergence of the adult and oviposition might be much longer than had been suspected. The writer therefore determined to look for the moths in the fields late in the summer.

After eggs had been found in the field, as recorded below, a very careful search on the soil in the cages was made, but without finding any eggs.

#### FIELD OBSERVATIONS IN 1915

During the season of 1915 the writer had an especially good opportunity to make observations in the field in connection with various trips to different parts of the State to aid farmers in the control of the cutworm and to collect material for the cages at Bozeman.

All through the summer, since some of the moths were still alive in the cages, the moths in the field or any clue that might indicate the time and place of egg laying were looked for. No moths were seen, except in or near fields which had been damaged, and even there none could be found a few weeks after the emergence of the moths. On July 16 and 17, moths were seen in great abundance at Willow Creek and were found hiding under clods of earth in the grain fields. At this time the last of the moths were just emerging, and some pupæ could yet be found. The same field was visited again on August 3, but no living moths could be found. Since the previous visit there had been a heavy rain, and by turning over clods of soil, many dead moths were found which had been trapped there. Since moths had been found so abundantly in this locality on July 16 and 17, it was thought that eggs also might be found. A careful, extended search, however, revealed none. Another search for eggs and young cutworms was made in this locality on September 21, but was entirely without results.

On July 17, the headlights of an automobile were used in the evening in an attempt to attract the moths in the field, but without much success.

In the early part of the evening one moth was caught, but a violent storm came up, preventing further search.

From this time until late in September, as recorded below, no moths were seen out of doors either at Willow Creek or elsewhere in the State. An electric-light moth trap was kept in use on fair, warm nights at Bozeman all summer, but no moths of this species were captured until late in September. Gillette<sup>1</sup> mentions what he considers to be two broods of the moths, one occurring between April 16 and July 10 and the other between September 13 and October 12. Wolley-Dod<sup>2</sup> records the capture of *C. auxiliaris*, *C. introjerens*, and *C. agrestis* in Alberta, Canada, in June and July and states that one specimen each of *C. introjerens* and *C. agrestis* was captured on September 9. One individual of *C. agrestis* was captured on May 19. Gillette also points out that he had been unable to find fully developed ova in the females of the first brood, though hundreds were dissected and examined, while dissected specimens of the fall brood, almost without exception, contained fully formed ova. This observation is of much importance and has been verified by the present author as stated elsewhere. It gives strong support to the conclusions of the present paper regarding the number of broods in the annual cycle. It also indicated that the ova are developed on food obtained as an adult rather than as a larva.

#### OBSERVATIONS ON THE EGG-LAYING HABITS OF THE SPECIES

On September 30 Assistant Entomologist J. R. Parker, of the Montana Station, while out on the college farm, saw noctuid moths flying in fair abundance. One was captured and brought in. On close examination it turned out to be a much-rubbed female of *C. auxiliaris*. Mr. Parker and the writer returned to the field at once to watch the moths. They were laying eggs in abundance directly upon the soil—not on plants, as had been expected. During the next few days extended definite observations on the egg-laying habits of the species were made on the college farm.

Several pieces of land had been recently plowed and harrowed. One field of 10 to 12 acres had been particularly well prepared for seeding some days earlier and was nearly free from vegetation, though a few grains and weed seedlings and grasses were to be found. The moths were seen in abundance on the soil in this field in fair weather day after day.

Egg laying was confined to the warm afternoons, and the moths were most active in the latter part of the day, from 3 o'clock until sunset. The mornings in October in Bozeman are generally quite cold, but a few warm forenoons occurred during which an unsuccessful attempt was made to observe egg laying in the field. By looking toward the west into

<sup>1</sup> Gillette, C. P. Some of the more important insects of 1903. *In* Colo. Agr. Exp. Sta. Bul. 94 (Tech. Ser. 6), p. 6. 1904.

<sup>2</sup> Wolley-Dod, F. H. Preliminary list of the Macro-Lepidoptera of Alberta, N.-W. T. *In* Canad. Ent., v. 37, no. 2, p. 49. 1905.

the sun's rays during the late afternoon many moths could be seen flying or walking along the surface of the soil. The moths were repeatedly seen to fly into the field from the grasslands or stubble fields adjoining and stop in the tilled field, where they immediately began to lay eggs. Several times they laid eggs on the bare earth of the roads on the college farm.

The moths also laid eggs in one field on the college farm just after it was plowed. Not once was a moth seen to lay eggs on any green plant or in any green field or stubble field; nor were any eggs found in such fields. Again and again, while watching the moths laying eggs at close range in the tilled field, they were seen to pass close by different kinds of vegetation without pausing. It was perfectly evident that they preferred to lay the eggs in the soil. By being careful one could witness the egg laying in detail by following along on hands and knees as a moth alternately paused to lay eggs and walked for a short distance. By far the greater number of the eggs were placed on the surface of the soil, often on small clods of earth, the moth standing on the clod and bending the abdomen downward and often tucking the eggs on the underside of the clod. Generally one or two eggs were laid on one spot, a few seconds being taken for the process. Sometimes but not always the moth frisked the tip of the abdomen back and forth sidewise repeatedly across the spot where the eggs were laid, thus dusting them and leaving a few scales from the clothing of the insect. The bright, glistening-white eggs are thus obscured. Some of the eggs were laid just beneath the surface of the soil. This could be done only where the soil had been pulverized, and in accomplishing it the ovipositor is thrust down through the surface of the soil and left for a few seconds. It is difficult to find such eggs under the surface of the soil, even when the spot is seen and the examination is made at once. One egg was found on a piece of dead straw. Generally only one to three were laid in one place, but in one case a moth deposited many eggs in soft soil within the space of a few square inches.

From the number of moths seen on the field and the number of days egg laying continued, it was roughly estimated that at least one or two eggs per square foot were laid in this field. By carefully searching a spot selected at random, eggs, almost certainly of this species, could be found. Four different persons, including the writer, have found eggs on the soil without having seen the moths deposit them. Both sexes were found among the moths captured in the field during the period of egg laying.

From these observations it can not be said that the eggs are necessarily always laid on bare or broken soil. In fact, it is almost certain that they are sometimes laid in abundance where newly plowed or newly harrowed soil is not available. A field of alfalfa badly infested with these cutworms was seen in Utah by the writer in May of the present year (1916), but no soil that could have been plowed last fall was anywhere in the vicinity. However, the stand of alfalfa was very scattering, leaving

much bare soil between the plants. A much-traveled road near by probably did not account for the presence of the worms. It seems more likely that the eggs were laid on the bare patches of soil in the alfalfa field.

There can be no doubt as to the specific identity of the moths that were observed, for many taken in the act of egg laying were carried into the insectary and placed in chimney cages, where they laid eggs in large numbers. Larvæ hatching from these eggs were reared to maturity, and the moths were obtained and all identified as belonging to this species. A few moths of other species were seen in the field during these observations, but none except *C. auxiliaris* were seen to deposit eggs.

#### ATMOSPHERIC TEMPERATURE DURING OVIPOSITION

No very definite temperature limits to oviposition can be stated. Thermometers were taken into the field and observed from time to time as the moths were being watched. The temperature during the rapid oviposition generally ranged between 55° and 70° F. A temperature of 60° to 70° at 4 or 5 o'clock p. m., with little or no wind stirring, insured great activity of the moths. When the sun set, the temperature dropped rapidly, and the moths sought shelter under clods and in cracks in the soil, very few being found still active in a temperature between 45° and 50°. Egg laying ceased at about 40°, though the moths were seen to fly at lower temperatures if disturbed. Whether or not the moths would continue active after dark if the temperature were favorable can not be stated.

#### DESCRIPTION OF THE EGG

Viewed from above, the egg is circular in outline; but when viewed from the side, it is very nearly elliptical, the shape varying from an ellipse only by being slightly flattened on the side on which it rests, which is opposite the micropile. It measures 0.62 mm. in diameter by 0.52 mm. in height. The color when the egg is first laid is white tinged with yellow, but before hatching the dark embryo shows through, giving the effect of a darker color. Surface markings on the chorion are very obscure. They are invisible under a hand lens magnifying 16 diameters. When viewed by reflected light under a compound microscope or under the high power of a binocular microscope, a very faint reticulation may be seen. This is more distinct in the shells of hatched eggs, in which the pattern often may be very clearly seen. No ridges radiating from the apex or upper part of the egg, such as may often be seen in noctuid eggs, have been found in this species.

#### EGG-LAYING HABITS IN CONFINEMENT

The moths taken into the insectary and put in cages were quite irregular in their egg laying. In general, two or three days were passed without laying eggs; then a large number were laid within a few hours, after which the moths soon died. Clover blossoms were placed in the cages,

and the moths were seen to be apparently feeding on the nectar. In the field during the same period the moths were seen to pause in their flight from field to field and visit blossoms of such plants as mustard and clover.

The various lots of eggs were allowed to hatch and the larvæ were reared in the greenhouse during the winter. Critical notes on instars and stadia were also made for later publication.

#### DURATION OF THE EGG-LAYING PERIOD

It is not probable that the writer observed the very beginning of the egg-laying period when, on September 30, the first moths were seen to be laying. It is altogether probable that the period began some days earlier, and there is no absolute evidence that egg laying had not been in progress for some weeks. It is quite clear, however, that the period closed about October 14, when a cold spell with rain occurred. When the weather cleared again, observations were resumed in the fields, but only a few scattering moths could be found, even though the temperature was favorable. October 8 was noted as the date on which the maximum activity of moths and egg laying was observed, and some eggs were laid as late as October 12. It may be safely stated that the egg-laying period is two weeks or more in duration.

#### NUMBER OF EGGS LAID

A detailed record of the number of eggs laid by individual moths was not made, as several circumstances in connection with the methods used interposed to make this difficult. The writer was influenced also by the fact that the moths had laid a part of their full number of eggs before being confined, which made it impossible to get complete data. The largest number actually counted was 252, all laid during one night, but this probably falls considerably below the actual maximum number. From this the number varies down to a very few, which may be accounted for in part by the moths having laid eggs in the field before being captured. The moth that laid 252 eggs died soon after and was dissected, the ovaries showing many immature ova.

#### DURATION OF THE INCUBATION PERIOD

The writer has complete records of the duration of incubation in 23 lots of eggs. A part of these were kept in the insectary and a part in an outdoor shelter. The minimum period recorded is 9 days, the maximum 21 days, and the average 16.77 days. The wide variation shown is striking and can probably be explained. The egg lots were kept during incubation in small tin boxes which were opened and examined daily. It was observed that many eggs were badly shrunken, and the dark embryos could be seen through the chorion. It was decided to add a very small amount of water to each box, as it was feared that the eggs would die from dryness. Accordingly, 1 to 3 drops of water were placed

in each of the boxes and the eggs hatched within a few hours. It seemed to be clear that incubation had been completed some days earlier in some of the lots, but that the young caterpillars had been prevented from issuing until sufficient moisture was present. Thus, some hatched in 9, while others hatched in 21 days. Those in the outdoor shelter hatched as soon as those inside. It is probable that 9 or 10 days is about the correct incubation period.

No field data on the incubation period are available. Repeated searching revealed no newly hatched caterpillars in fields where numerous eggs were known to have been laid. It is very interesting to note also that no larvæ could be found this spring in the field on the college farm where the eggs are known to have been laid last fall.

#### LARVAL FEEDING IN THE FALL

Only scattering records of larvæ in the fall are available, but these are of considerable interest. In the fall of 1906 the very small larvæ of this species did some damage in the northern part of Gallatin Valley. Several lots of the larvæ were received at the Experiment Station in November, and reports of their occurrence had reached it in October. One lot was reared to the adult condition. This is the only case known here in which the larvæ have attracted the attention of the farmers in the fall, and in this case the knowledge of their presence served as a useful warning of their coming in destructive numbers the next spring. The fall of 1906 was unusually dry and warm, the mild weather continuing until late. The larvæ continued feeding until December 6.

On November 4 and 5, 1915, an assistant was able to find larvæ in nearly every field of grain examined in Fergus County. They were not very abundant, but were easily found by the holes eaten in the leaves. At this time the worms were very small, probably in the second instar. Cold weather occurred soon afterward and larval feeding must have ceased. On April 10, 1916, some of the same fields were visited again and the larvæ were still very small. They certainly were very much smaller than on the same date in 1915. It is clear that there is a considerable variation in the size reached before winter sets in and, hence, in the size of the larvæ in the spring.

#### HIBERNATION OF THE INSECT

From the foregoing and from Johnson's observations<sup>1</sup> it is clearly evident that the insect hibernates as a partly grown larva.

It has been stated above that in the fall of 1906 the larvæ fed until December 6. The feeding of the caterpillars ceased with the coming of a snowstorm. A field which had been visited only a few days before and which was known to contain many larvæ was examined after this storm. The snow was swept away with a broom, and the larvæ were found on

<sup>1</sup> Johnson, S. A. Cutworms. In Colo. Agr. Exp. Sta. Bul. 98, p. 18. 1905.

and near the surface of the soil in a torpid condition. When taken into the hand, they immediately warmed up and began to crawl. There was apparently an absence of any quiescence other than torpor induced by cold.

#### DURATION OF LARVAL FEEDING IN THE SPRING

In Montana the larvæ resume activity with the beginning of the growth of vegetation, which is generally in the latter part of March or early in April. In 1910 the first larvæ from the field were sent in on April 24. In 1915 the first to be received at the Experiment Station came on April 2. By April 15 the station was receiving many urgent requests for information regarding control, indicating that the worms were very active. They continued in abundance in the field until about the third week in April and gradually disappeared until early in May, as indicated by many observations in the field and by the correspondence. In 1915 several lots of larvæ from various parts of the State were reared in the insectary and began pupating on April 22 and continued until May 19, when the last had transformed. The greater part of these had pupated by May 10.

In general, the occurrence of the larvæ in "armies" may be said to extend from April 1 to May 1.

#### PUPATION AND EMERGENCE OF ADULTS

From the notes of the writer on the rearing and more especially from information regarding the disappearance of the larvæ in the field it is clearly evident that by the last week in April in average years the larvæ are rapidly disappearing. Pupation takes place in an earthen cell about 2 inches under the surface of the soil. The pupa always rests with the anterior end uppermost, and the molted skin lies beside it.

The duration of the pupal stage, as indicated by the rearing records of many isolated individuals in the insectary and not including rearings conducted during the winter months in an artificially heated greenhouse, varies from 43 to 63 days and averages 54.7 days. From field observations the duration of this stage has been determined to be approximately 60 days. The first week in May clearly marks the height of pupation out of doors. On July 16 and 17 fresh moths were found in great abundance in the fields at Willow Creek, while only a very few pupæ could be found. It may be safely assumed that the last of the moths were emerging about this date, and the height of emergence was during the first week in July.

The writer several times has noted that a small advantage of temperature markedly hastens the appearance of the moths of this insect. If kept in a cool place, the emergence of the moths may be greatly delayed. That the time of emergence varies in different seasons is shown by the fact that on July 8, 1910, in attempting to get pupæ 8 miles west of Bozeman it was found that the moths had all emerged. This was an early, dry season. It is quite clear that the first of the moths appear in June.



## NUMBER OF BROODS

From the foregoing observations it is clearly evident that the army cutworm passes through but one annual life cycle. There is not time enough for a second brood to occur between the appearance of adults in the early part of July and the laying of eggs about October 1. Nine days is the shortest incubation period the writer has noted. The only accurate data of the writer regarding the duration of the larval stage were obtained by rearing to maturity in a heated greenhouse in the winter of 1915-16 the various lots of eggs laid by moths in October, 1915. It is believed that the larval period in the greenhouse was probably shorter than it would have been out of doors even in the summer time. The longest period recorded was 118 days, the shortest 96, and the average was 104.06 days. As stated above, the shortest pupal period secured in the indoor rearings was 43 days, which added to the minimum periods gives 148 days, while from July 1 to October 1 there are only 92 days, thus leaving a difference of 56 days. Moreover, no larvæ have been found at any time during the summer which might belong to a second brood.

Observations given in previous paragraphs which have a bearing on this question may be recapitulated. Moths caught in June and July are bright and fresh; those caught in the fall are rubbed and faded. The ovaries are immature in July, while in September and October they are mature. Moths placed in cages at Bozeman in July and given honey water and clover blossoms daily lived until late in September.

If it be assumed that the brood of moths emerging in June and July live over until fall, meantime growing ova, then the 12 months of the year are all accounted for in one life cycle of the insect.

## SUMMARY

From the foregoing observations the life history of the army cutworm (*Chorizagrotis auxiliaris*) may be summarized as follows:

- (1) Egg laying was observed from September 30 to October 12, but possibly occurred for some weeks previous to September 30.
- (2) The moths deposit the eggs directly upon the bare soil.
- (3) The incubation period is about nine days indoors, but hatching may be delayed by lack of sufficient moisture.
- (4) The larvæ feed for a variable period in the fall which terminates with the onset of winter.
- (5) The insect hibernates as a partly grown larva.
- (6) Activity is resumed by the larvæ with the beginning of plant growth in the spring.
- (7) The larvæ feed until about the first week in April, when they enter the earth for pupation.
- (8) The moths emerge from the latter part of June to the middle of July.
- (9) The moths live over until fall, growing ova on food obtained as adults.
- (10.) The army cutworm is single brooded in Montana.



# APHIDOLETES MERIDIONALIS, AN IMPORTANT DIPTEROUS ENEMY OF APHIDS

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## INTRODUCTION

The economic importance of *Aphidoletes meridionalis* Felt was established when the larvæ of this cecidomyiid fly were first observed by the writer at La Fayette, Ind., on June 29, 1912, destroying large colonies of *Aphis setariae* and *Hyalopterus pruni* on plum (*Prunus* spp.). Subsequent observations in the States of Iowa, Wisconsin, Illinois, Michigan, and Indiana emphasize its value as an efficient agency in the natural control of Aphididae.

While most of the data reported in this paper were obtained during July and August, 1912, it was impossible to obtain a specific determination of the species from specimens used in the experiments at that time, but during the past season (1915) Dr. E. P. Felt has kindly determined it as *Aphidoletes meridionalis* from living adults reared from larvæ collected in the same locality and attacking the same kinds of aphids as those used in the experiments of 1912. *Aphidoletes meridionalis* was described by Dr. Felt in 1908 from adults reared from larvæ predacious on the tulip-tree aphis (*Macrosiphum liriodendri* Monl.).<sup>1</sup>

## ECONOMIC IMPORTANCE, NATURAL CHECKS, AND APHIDS ATTACKED

The fact that each larva may destroy dozens of aphids and that these flies are remarkably prolific makes this predator very important and valuable. Many instances were observed where aphid colonies were apparently completely destroyed. For example, on June 6, 1915, the undersides of leaves of catnip (*Nepeta cataria* L.) in the writer's yard were completely covered with *Aphis gossypii*, and at that time *Aphidoletes meridionalis* was just making its appearance in numbers, the eggs and larvæ up to half or possibly two-thirds grown being abundant. A week later (June 13) very few aphids remained, and most of the predacious larvæ had made cocoons on the undersides of leaves between the leaf veins or on the ground at the base of plants, and a few days later only very rarely could a live aphid be found. A few syrphid larvæ, an occasional coccinellid larva or adult, and some aphidiine parasites were present, but the control of the aphis was apparently due entirely to *Aphidoletes meridionalis*.

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<sup>1</sup> Felt, E. P. Studies in Cecidomyiidae II. In 23d Rpt. State Ent. N. Y., 1907, p. 384, 397. 1908. (N. Y. State Mus. Bul. 124).

Although the fly is prolific and constitutes an effective check to the increase of aphids under favorable conditions, the adults are very frail and easily destroyed by unfavorable weather conditions, such as beating rains. They do not, as a rule, make their appearance in appreciable numbers until the latter part of May and probably can not, therefore, be considered as being so generally reliable as a natural means of control as are the hymenopterous enemies belonging to the subfamily Aphidiinae.

This cecidomyiid is a general feeder, attacking almost any species of aphid available, but more often feeding on those which live gregariously upon their hosts. The writer's records show that it attacks the following species: *Aphis asclepiadis* Fitch., *A. avenae* Fab., *A. cardui* L., *A. gossypii* Glov., *A. helianthi* Monl., *A. maidis* Fitch *A. setariae* Thos., *Chaitophorus negundinis* Thos., *Hyalopterous pruni* Fab., *Macrosiphum granarium* Kibby, *M. pisi*, Kalt. *M. sonchella* Monl., *Myzus persicae* Sulz., *Phorodon humuli* Schr., *Rhopalosiphum sonchi* Oestl., *Siphi flava* Forbes, *Siphocoryne pastinacae* L., and *Toxoptera graminum* Rond.

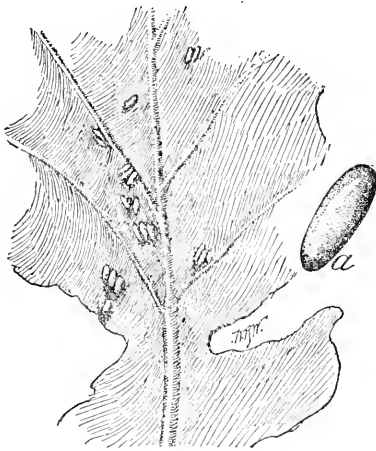


FIG. 1.—*Aphidoletes meridionalis*: Eggs in situ on leaf of rape; a, egg, greatly enlarged.

#### HISTORICAL SUMMARY

Aside from systematic discussions, very little has been written about *Aphidoletes meridionalis*. There can be no doubt that the larvæ predacious on *Macrosiphum pisi* and referred to by Fletcher in his report for 1900<sup>1</sup> as a species of *Diplosis* were *Aphidoletes meridionalis*, and this seems to be the first authentic record in economic literature. A short account of the habits of probably the same species as the one under discussion is given by Webster and Phillips,<sup>2</sup> who refer to it as an enemy of *Myzus persicae* and of the spring grain aphid or "green bug" (*Toxoptera graminum*) and predict that it may possibly become an important factor in the control of *T. graminum*. The writer<sup>3</sup> has referred to this species as an active enemy of the pea aphid (*Macrosiphum pisi*) and other writers have barely referred to it as predacious on aphids.

<sup>1</sup> Fletcher, J. T. Report of the entomologist and botanist. 1900. In Canada Exp. Farms Rpts. 1900, p. 212. 1901.

<sup>2</sup> Webster, F. M., and Phillips, W. J. The spring grain-aphid or "green bug." U. S. Dept. Agr. Bur. Ent. Bul. 110, p. 133. 1912.

<sup>3</sup> Davis, J. J. The pea aphid with relation to forage crops. U. S. Dept. Agr. Bul. 276, p. 54. 1915.

LIFE HISTORY AND DESCRIPTIVE NOTES

The eggs (fig. 1) are very small, elliptical oval, chrome orange in color, paler at the extremities, and measure 0.104 mm. in width and 0.313 mm. in length. They are laid in clusters of from 1 to 12 on foliage amongst a colony of aphids or may be deposited on the dorsum of the aphid itself, as many as 7 having been noted on a single aphid. The number of eggs laid by individual females was determined in two cases (Table I), and it will be noticed that these females laid 116 and 125 eggs each, respectively. The cages used for obtaining eggs were of the ordinary "chimney" type and the results certainly were not above normal and more likely were below normal. The exact length of the egg stage was not accurately determined, but from the approximate records given in Tables I and II and from some more exact miscellaneous records the length of the egg period averages about three days.

TABLE I.—Records of eggs of two individual females of *Aphidoletes meridionalis*; La Fayette, Ind., August, 1912

Cage No.	Date male and female were introduced into cage.	Date first lot of eggs were counted.	Number.	Hatching and rearing records.	Date second lot of eggs were counted.	Number.	Hatching and rearing records.	Date third lot of eggs were counted.	Number.	Hatching and rearing records.
7654(4)	1912- Aug. 1	Aug. 3	55	Eggs hatching Aug. 6; adults Aug. 22-23.	Aug. 5	30	Eggs hatching Aug. 8; adults Aug. 22-24.	Aug. 7	11	Adults Aug. 25-26.
7654(5)	<sup>a</sup> Aug. 2	Aug. 5	17	Eggs hatching Aug. 8; adults Aug. 24.	Aug. 7	22	Adults Aug. 23-24.	Aug. 9	38	Adults Aug. 25.

Cage No.	Date male and female were introduced into cage.	Date fourth lot of eggs were counted.	Number.	Hatching and rearing records.	Date fifth lot of eggs were counted.	Number.	Hatching and rearing records.	Date of death of Aphidoletes female.	Total number of eggs.
7654(4)	1912- Aug. 1	Aug. 11	20	Adults Aug. 26-27.				Aug. 11?	116
7654(5)	<sup>a</sup> Aug. 2	Aug. 12	35		Aug. 15	11		Aug. 15	123

<sup>a</sup> This pair was observed in copula at 7.30 p. m. on Aug. 2.

Immediately upon hatching the larva attacks the most convenient aphid, and at this stage of its life more often pierces the body of its host from beneath, usually between the legs. After sucking the body fluids from the first aphid and killing it, the larva leisurely moves to another, this operation being continued until it becomes full grown. The larva always seems to move about cautiously, at the same time quickly thrusting its tongue-like anterior end in and out and to all sides much as does a syrphid larva. When it locates its host it thrusts its proboscis into the

aphid and sucks the body fluids until the aphid is dead and more or less shriveled. The victim seldom notices the presence of the larva, judging from outward indications. After the larva becomes one-third grown it usually punctures the aphid at one of the articulations of the legs, a favorite

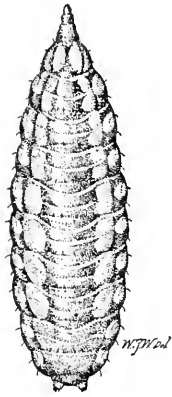


FIG. 2.—*Aphidoletes meridionalis*: Larva, dorsal view. Greatly enlarged.

point of attack being at the membranous joint connecting the tibia and femur (Pl. CX, fig. 2). If the larva attacks the aphid at an articulation as above described, the latter seldom notices the attack; but if the proboscis of the larva touches the wrong places, the aphid kicks about more or less for a few seconds. As a rule, several minutes are required for the larva to pump out most of the body juices of the host, but this time varies, depending upon the relative sizes of the larva and its host. The aphid is often discarded by the larva soon after it has been killed and long before it has been sucked dry.

To the naked eye the larva of *Aphidoletes meridionalis* (fig. 2) closely resembles such common cecidomyiids as the cloverleaf midge (*Dasyneura trifolii* Loew), but differs slightly in coloration, being usually of a pale orange, varying from pale pinkish to a rather deep orange, and when mature measures approximately 3 mm. in length. The length of the larval period varies, depending upon the temperature and food supply; but according to observations of the writer it is between 7 and 11 days.

When fully mature the larva spins a loose cocoon of silk mingled with aphid remains, attaching it to the leaf between the veins; or it descends to the ground and at or near the surface spins its cocoon (fig. 3), incorporating with it particles of dirt and trash. The larva pupates shortly after constructing the cocoon. The pupa (fig. 4), which is of an orange color, resembles other related cecidomyiids; it measures 2 mm. in length and its cocoon is 2.25 mm. long and 1.125 mm. wide. The length of the pupal stage varies, according to observations, between 6 and 9 days.

The adult (Pl. CX, fig. 1) may be popularly described as a small, frail midge, much resembling, to the casual observer, (*Dasyneura*) *Neocerata rhodophaga* Coq. the destructive rose midge, or the clover-seed midge (*Dasyneura leguminicola* Lintn.). Its length is approximately 1.4 mm. for the male, and 1.8 mm. for the female; the body is pale and the abdomen has a decided pinkish tint. Copulation and egg laying seem to occur



FIG. 3.—*Aphidoletes meridionalis*: a, Cocoon formed on surface of ground; b, cocoon formed on a leaf blade.

at night—at least they have been observed by the writer only at night, although the cages were examined much more frequently during the day.

Egg laying continued over a period of about 10 days in "chimney" cages, and the length of life of the nidge under the same conditions was about 14 days. Several unsuccessful attempts were made to induce unfertilized females to lay eggs, although fertilized females laid eggs readily, indicating that this species is probably not parthenogenetic.

As will be seen from Table II, the total length of the life cycle from egg to adult varied, in the region in which it was studied, from 15 to 29 days, the average normal life cycle being about 18 to 20 days. The seasonal number of complete generations has not been determined, but there are evidently at least six complete generations annually, the winter being passed as larvæ and possibly also as pupæ within the cocoons.



FIG. 4.—*Aphidoletes meridionalis*: Pupa, lateral view. Much enlarged.

TABLE II.—Length of life cycle of *Aphidoletes meridionalis*; La Fayette, Ind., July-August, 1912

Cage No.	Eggs laid—	Eggs hatched—	All larvæ finished feeding—	Cocoons noticed—	Adults issued—	Total life cycle.	
						Minimum.	Maximum.
	1912.					<i>Days.</i>	<i>Days.</i>
7654(1)c.....	July 15-16...	July 17-18...	.....	.....	Aug. 5-14...	20	30
7654(1)d.....	July 17-18...	July 20.....	.....	July 25-29...	Aug. 5-13...	18	27
7654(3)a.....	Aug. 1.....	Aug. 5-6...	Aug. 13.	.....	Aug. 21.....	20	
7654(4)a.....	Aug. 9-11...	.....	.....	.....	Aug. 26-27..	15	18
7654(4)a.....	Aug. 1-3...	Aug. 6.....	Aug. 15.	.....	Aug. 22-23..	19	22
7654(4)b.....	Aug. 3-5...	Aug. 7-8...	do.....	.....	Aug. 22-24..	17	21
7654(4)c.....	Aug. 5-7...	.....	Aug. 14.	.....	Aug. 25-26..	18	21
7654(5)a.....	Aug. 3-7...	Aug. 7-8...	.....	.....	Aug. 24.....	19	21
7654(5)b.....	Aug. 5-7...	.....	.....	.....	Aug. 23-24..	16	19
7654(5)c.....	Aug. 7-9...	.....	.....	.....	Aug. 25.....	16	18
7654(2)a.....	July 15-16...	July 18.....	July 25..	July 24-26..	Aug. 1-13...	16	29
7654(2)b.....	July 16-17...	.....	.....	July 26.....	Aug. 4-7....	18	22

PLATE CX

*Aphidoletes meridionalis:*

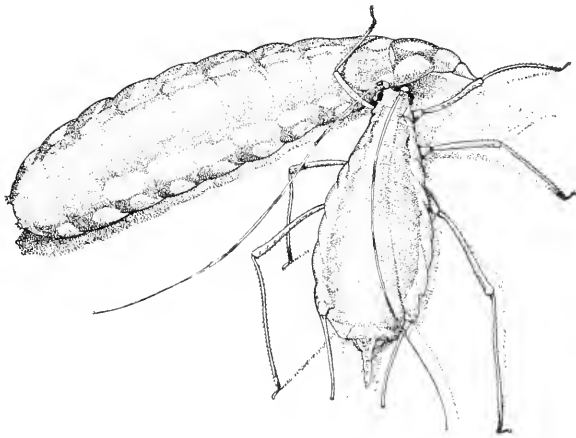
FIG. 1.—Adult female: *a*, Antenna of male, showing structure; *b*, tip of male abdomen. Greatly enlarged. (Redrawn after Webster and Phillips.)

Fig. 2.—Larva attacking a pea aphid (*Macrosiphum pisi*). (From Davis.)





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# INFLUENCE OF BARNYARD MANURE AND WATER UPON THE BACTERIAL ACTIVITIES OF THE SOIL

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## INTRODUCTION

The application of barnyard manure to a soil brings about a far-reaching change within the soil. It has been found that, as an average, 1 ton of barnyard manure contains 10 or 12 pounds each of nitrogen and potassium and 2 or 3 pounds of phosphorus. It also carries other substances of less importance which may be directly utilized by the growing plant or which may react with substances within the soil, changing their solubility. This direct and indirect nutritive value of a manure is not its only function, for it changes greatly the physical structure of the soil. It improves the tilth of a clay soil by increasing the granulation within it, while in a sandy soil it tends to bind the particles together, making it less porous. Each of these changes react upon the water-holding capacity and the capillarity of the soil, greatly altering the aeration of the soil and with it the temperature.

The biological changes which the manure produces in the soil, especially when small quantities are added, may be more far-reaching than either the chemical or physical changes which it produces. Every pound of manure carries with it to the soil millions of bacteria. Many of these will find the new conditions unsuited for their growth, but some will continue to multiply, and in so doing not only will decompose the constituents of the manure but also will greatly alter other organic and inorganic substances of the soil. Hence, the bacterial content of the soil is changed both quantitatively and qualitatively. There are added with the manure many new species, and the changed physical and chemical conditions of the soil due to the manure will greatly modify those already present, for the microflora and fauna originally present in the soil were due to specific properties of the soil.

This changed flora and fauna will in turn change the chemical and physical properties of the soil still more. Acids are generated, which react with insoluble constituents, rendering them soluble. Gases are formed, which change the air within the soil; and in these reactions heat is generated, thus changing the temperature of the soil. The metabolism of the bacterial cell requires nutritive substances, among which are water

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and the elements essential to plant growth. Some soluble constituents will be changed to insoluble and some inorganic to organic. All of these changes must be reflected in the yield of the crop produced.

This investigation was undertaken to throw more light on some of these changes, especially the influence of manure in the presence of varying quantities of water upon the bacterial activities of the soil, and it may be seen by an examination of the more important literature on the subject that with respect to the control of both manure and moisture this experiment is unique.

#### HISTORICAL REVIEW

That the addition of manure to a soil increases the number of bacteria has been shown by Remy<sup>1</sup> (37, p. 660-733) and Fischer (13, p. 358). Caron (6) found that the number of bacteria present depends not only upon the manure added but upon the cultural methods and the crop grown upon the soil. Fabricius and Von Feilitzen (12) found that bacteria increased in the soil on the addition of manure and that a direct relationship existed between the temperature of a soil and the number of bacteria found in it. That the temperature of the soil is influenced by the addition of manure is shown by Wagner (47), who observed that manure increased the temperature of soil from 1 to 2.8 degrees centigrade, depending on the kind and condition of manure added. Troop (44) noted an average increase of 5 degrees in temperature of soil receiving 25 tons per acre of manure over unmanured soil. Petit (35), however, claimed that, while there was at first an increase in the temperature of manured soils, later it became lower than the unmanured. Stigell (41) concluded that bacteria under favorable conditions for development retarded the conduction of heat in soils and thereby reduced the temperature changes due to the variation in the outside temperature. This in a way might neutralize the effect of manure, for Hecker (20) found that while the temperature of soil to which well-rotted manure had been added was higher than adjacent unmanured soil during the day, the opposite was true during the night. Grazia (17) stated that manures greatly increase the temperature of the soil. King (26) found that a definite increase in bacterial activity occurred with increased temperature, but that an excessive moisture content greatly reduced the number of bacteria in a soil. Engberding (11) claimed that manure increased the number of bacteria in a soil, but he considered that the moisture content had a greater influence on numbers than did temperature. That the moisture content greatly influenced bacterial activity was shown by Dehérain and Demoussy (9), who found that the bacterial action of a soil was at its maximum when a rich soil contained 17 per cent of water, but that it decreased if the proportion of water fell to 10 per cent or rose to 25 per

<sup>1</sup> Reference is made by number to "Literature cited," p. 923-926.

cent. With soils less rich in humus a somewhat higher proportion of water was necessary to retard oxidation to any marked degree. In a manured soil the coarse manure tended to cause the surface soil to dry out, while fine manure prevented evaporation. King (25) observed that manured land contained more moisture throughout the year than unmanured soil, and this was reflected upon both the bacteria and the crop. The bacteria themselves may play a small part in this difference in moisture content, as was shown by Stigell (42), who found that bacteria decreased the speed of evaporation of water from Petri dishes. Hiltner and Störmer (24) claimed that the addition of manure to a soil brought about a marked increase in the number of bacteria. The temperature, cultural methods, and crop had an influence, but it was not nearly so pronounced as that produced by the manure. Dafert and Bolliger (8) stated that the difference in moisture did not have to be great to produce a great change in the oxidation going on in the soil, for a distinctly measurable difference was noted when the moisture varied 1 per cent.

Brown (4), in a study of the influence of manure on the bacterial activities of a loam soil, found that applications of manure up to 16 tons per acre increased the number of bacteria and also the ammonifying and nitrifying powers of the soil. The greatest increase in the processes was brought about by small applications of manure, 8 to 12 tons to the acre. He observed a close relationship between the ammonifying powers of the soil, the bacterial content, and the crop produced on the soil.

Temple (44) stated that the addition to a soil of 10 tons of cow manure per acre greatly increased the number of bacteria in the soil, but that a greater increase occurred when a sterilized manure was applied. This, however, is not in keeping with the results obtained by other investigators, for Hellström (22) concluded that manures possessed a fertilizing effect aside from the quantities of fertilizer constituents contained within them; and this, he claimed, is their great bacterial content. And Stoklasa (43) found that manure increased the bacterial content and activity of a soil and was greater with small, frequent applications of manure than with large applications made at longer intervals. Moreover, Lipman and others (31) observed that the bacteria conveyed to soil in small quantities of manure were valuable in bringing about a more rapid decomposition of a green-manure crop, while Briscoe (3) said that a direct relationship existed between the organic matter added to a soil and the bacterial count and that a light dressing of manure with green manure produced a marked effect upon both the crop and the bacterial count. Bacterial cultures added with the green manure gave just as pronounced an effect as did the stable manure. Lemmermann and Einecke (29), however, obtained no increase on adding stable manure with green manure. This may be due to the different kind of manure used, for Emmerich and others (10) claimed that a more favorable effect

was obtained from the use of well-rotted manure than fresh manure. This, they claimed, was due to the production in the latter of formic, acetic, and butyric acids, indol, skatol, and hydrogen sulphid, which are toxic to the plant. Under some conditions the large quantities of carbon dioxid liberated from the rapidly decomposing fresh manure may be valuable in rendering soluble plant food. Bornemann (2) found that soil constantly supplied with carbon dioxid through a pipe buried in the ground gave an increase in yield of 12.2 per cent over the crop grown on untreated soil. Wollny (52) has shown that manure greatly increased the carbon-dioxid production in a soil.

Moll (33) claimed that the season of the year and not the kind of fertilizer used, nor even the weather conditions, is the principal factor in determining the peptone decomposition, nitrification, and nitrogen fixation of a soil. According to Wohltmann, Fischer, and Schneider (51), ammonification, nitrification, and nitrogen fixation were all more or less increased by the application of manure. Lipman (30, p. 135) found that the peptone-decomposing power of a soil was greatly increased by the application of manure. Heinze (21) found that manure was especially beneficial to the nitrifying organisms. Warrington (48) reports that much more nitric nitrogen was found in the soil of plots which had received annually for 38 years a dressing of 14 tons of manure to the acre than in any of the other manured or unmanured plots. While Stevens (39) found that nitrification was much more active in manured than in unmanured soil, Frankfurt and Duschechkin (14) observed an increase in nitrification only on those manured plots on which the yield had increased. Velbel (46) has shown that the chief factors controlling nitrification in fallow soil were the humus and the humus-nitrogen content of the same, the nitrification having increased directly with the humus. He noted, however, a certain amount of denitrification at first, but later in the summer nitrification became more rapid on the manured than on the unmanured soil, the effect of the manure being still perceptible after four years. Some investigators (23, 36, 50) have reported a reduction of nitrates, but the quantity of manure applied was excessive, or else of a very coarse nature, or the soil very poorly aerated. Barthel (1) found that nitrification did not take place in the presence of soluble organic matter, but he considered it unlikely that sufficient quantities of soluble organic constituents occurred in normal agricultural soils to interfere greatly with nitrification. Niklewski (34) claimed that nitrification occurred in solid stable manure when there was not much liquid present. He stated that on the first day some nitrite bacteria were present and at the end of four weeks there were 10,000 per gram. Associated with these were nitrate bacteria which were identical with those isolated by Winogradsky. Millard (32), however, was unable to find many nitrifying bacteria in manure.

Many of the cases in which individuals have reported a disappearance of nitrates in soil are due to synthetic reactions, the nitrates being built up into complex proteins. For Gerlack and Vogel (15) have shown that there are several varieties of bacteria in the soil which have the power of converting ammonia, nitrites, and nitrates into insoluble proteins.

The processes of ammonification, nitrification, and nitrogen fixation, being due to the action of micro-organisms, are intimately associated with the moisture content of the soil; hence, we find in many cases this is the limiting factor. Guistiniani (16) found in sandy soil that the rapidity of nitrification of ammonium sulphate was directly proportional to the amount of moisture present when this varied from 0 to 16 per cent. While Roche (38) has shown that irrigation supplying from 15 to 25 per cent of water to a soil furnished the most favorable conditions for nitrification, Coleman (7) found nitrification most active in a loam soil with a moisture content of 16 per cent. It was greatly retarded when the water content was reduced to 10 per cent or raised to 26 per cent. It is also interesting to note that he found that with a high moisture content soluble organic matter became injurious to nitrification.

The nitrogen-fixing organisms would also be influenced by the water content, as shown by Warmbold (49), who stated that when the water content went below 10 per cent there was no nitrogen fixation and in some cases there was a decided loss of nitrogen. Krainskiĭ (27) said that nitrogen fixation was at its height in soils containing fairly small quantities of water. Later he (28) stated that the higher the humus content the larger the water content of the soil required for optimum nitrogen fixation. Increasing the organic matter of the soil was not found to increase nitrogen fixation, although there was an increased bacterial activity. Hanzawa (18) found that the humus of stable manure could be used as a source of energy by some nitrogen-fixing bacteria.

#### PLAN OF EXPERIMENT

The plan of the experiment is such that it can be divided into three parts. The first deals with the bacterial activities of a soil receiving a definite amount of manure and measured quantities of irrigation water and kept fallow in pots under vegetation house conditions. In this the moisture content could be accurately maintained by the weekly weighing and the replacing of lost moisture. The variation in temperature and moisture of this series would not be as great as it would be under field conditions. The second part deals with the bacterial activities going on in a soil under field conditions, the soil receiving known quantities of manure and water but kept fallow. The third part deals with soil of the same field under irrigated conditions and manurial treatment the same as the second part, but producing a crop.

## COMPOSITION OF SOIL

The investigation was conducted either on soil from the Greenville Experiment Farm or on the farm itself, which is situated 2 miles north of Utah Agricultural College. The soil represents a type found in large areas in the Great Salt Lake Basin. It is of a sedimentary nature, being derived from the weathering of the mountain range near by, which consists largely of limestone and quartzite deposited by the streams as they flowed into the now extinct Lake Bonneville. The soil is situated at the foot of the main delta thus formed and consists of fine sand and coarse silt of fairly uniform chemical and physical composition to a great depth. The chemical and physical analysis of the soil is given in Table I. The chemical analysis was made according to the official methods of the Association of Official Agricultural Chemists,<sup>1</sup> while the physical analysis was made by means of the Yoder soil elutriator.

TABLE I.—*Chemical and physical composition of the soil of the Greenville (Utah) Experiment Farm*

Chemical composition.		Physical composition.	
Constituent.	Per cent.	Constituent.	Per cent.
Insoluble residue . . . . .	41.46	Coarse sand . . . . .	0.21
Soluble silica . . . . .	.62	Medium sand . . . . .	9.63
Total . . . . .	42.08	Fine sand . . . . .	30.04
Potash (K <sub>2</sub> O) . . . . .	.67	Coarse silt . . . . .	32.25
Soda (Na <sub>2</sub> O) . . . . .	.35	Medium silt . . . . .	12.30
Lime (CaO) . . . . .	16.88	Fine silt . . . . .	6.25
Magnesia (MgO) . . . . .	6.10	Clay . . . . .	7.62
Oxid of iron (Fe <sub>2</sub> O <sub>3</sub> ) . . . . .	3.93	Moisture . . . . .	1.60
Alumina (Al <sub>2</sub> O <sub>3</sub> ) . . . . .	5.64	Soluble and lost . . . . .	.10
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> ) . . . . .	.41	Specific gravity . . . . .	2.67
Carbon dioxid (CO <sub>2</sub> ) . . . . .	19.83	Apparent specific gravity . . . . .	1.23
Volatile matter . . . . .	5.60	Water-soluble salts . . . . .	.06
Total . . . . .	100.69		
Humus . . . . .	.53		
Nitrogen . . . . .	.139		

The soil has been analyzed to a depth of 10 feet and was found to be very similar in both chemical and physical composition to that given in Table I. There were, however, slightly greater quantities of acid-soluble material in the lower foot sections. The humus and nitrogen of the deeper soil was slightly less than in the first foot. The physical composition is practically the same to a depth of 10 feet. The soil is exceptionally rich in phosphorus and potassium, but low in nitrogen and humus. The calcium and magnesium contents are exceptionally high and one may conclude that for this reason the soil is unproductive; but just the reverse is true, for the soil is very fertile and even with its low nitrogen and humus content produces excellent crops.

<sup>1</sup>Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted 1912.



## METHOD OF SAMPLING THE SOIL

All possible precautions against the contamination of one sample by another were taken in collecting them. The surface soil to a depth of half an inch was scraped off by means of a sterile spade. A hole 12 inches deep was dug, and a slice of soil to this depth was taken from the side of the hole and placed in a sterile mixing pan. This process was repeated from four or five places in the field and then the contents of the pan carefully mixed by means of a sterile spatula. From this composite sample a representative portion, about 5 pounds of soil, was placed in a sterile ore sack and conveyed to the laboratory for analysis.

Before each sampling, the spade, mixing pan, and spatula were all carefully sterilized by heat from a plumber's torch, thus preventing the transfer of organisms from one soil to another. The samples were immediately transferred to the laboratory, partly air-dried in the dark, and then ground in a sterile mortar, all coarse rock being removed. The analysis was begun in all cases within 24 hours of the time of taking samples.

## METHODS OF SOIL ANALYSIS

The number of organisms were determined by growing on modified synthetic agar having the following composition:

- 1,000 c. c. of distilled water.
- 10 gm. of dextrose.
- 0.5 gm. of dipotassium phosphate ( $K_2HPO_4$ ).
- 0.2 gm. of magnesium sulphate ( $MgSO_4$ ).
- 2 gm. of powdered agar per 100 c. c. of media.

After the samples of soil had been carefully mixed by shaking 100 gm. were weighed on a sterile watch glass, using a small sterile spatula. This soil was transferred to 200 c. c. of sterile water and shaken for one minute, 1 c. c. of this suspension transferred to 99 c. c. of sterile water, and the dilution continued with 9 c. c. of sterile water. The plates were made so as to give a dilution of 1 to 20,000 and 1 to 200,000. They were incubated at 28° C. for four days and then counted. No attempt was made to differentiate between bacteria and molds, but all were listed together as total numbers of colonies.

The ammonifying power of the soil was determined by weighing 100-gm. portions of the soil and 2 gm. of dried blood into sterile tumblers and covering them with Petri dishes. The dried blood was thoroughly mixed with the soil by means of a sterile spatula and the water content made up to 18 per cent with sterile water. The samples were incubated at 28° to 30° C. for four days and the ammonia determined by transferring to Kjeldahl flasks with 250 c. c. of distilled water, adding 2 gm. of magnesium oxid and distilling into *N/10* sulphuric acid. The determinations were all made in duplicates and compared with sterile blanks.

The nitrifying power of the soils was determined in tumblers, like the ammonifying power, except that they were incubated for 21 days. The moisture content was made up weekly to the initial 18 per cent.

At the end of the incubation period each soil was transferred with 250 c. c. of distilled water to a 1-pint Mason fruit jar. Two gm. of powdered lime were added and the jar placed in the shaking machine for 10 minutes, after which it stood in the closed jar until clear. This never required over two hours. At the end of this time an aliquot part, 100 c. c., was measured into a flask and the nitrates determined by the aluminum reduction method (5).

The nitrogen-fixing powers of the soil were made by weighing 5-gm. portions of the soil into 500 c. c. Erlenmeyer flasks containing 100 c. c. of Ashby solution. These, together with sterile blanks, were incubated for 18 days and then the total nitrogen determined by the Kjeldahl method. All determinations were made in triplicate.

#### POT EXPERIMENTS

Dry soil, to a depth of 12 inches, was taken from one of the unmanured plots of the Greenville Farm and very carefully mixed and used as the soil for the pot experiments. This soil, together with the required quantity of well-rotted barnyard manure, was packed into the pots. Moisture determinations were made upon the mixtures and then sufficient water added to make up to the required moisture content. The pot and contents were weighed and the moisture content made up weekly to the initial content. The pots were kept on shelves within the building for four months, and then the various determinations were made on each sample as outlined. The temperature of the soil was taken each time before making it up to the moisture content. The manure was applied at the rate of none, 5, 10, 15, 20, and 25 tons to the acre. An acre of soil was considered as weighing 2,000,000 pounds. Each ton of the manure contained 738 pounds of dry matter, 3.04 pounds of phosphorus, 13.70 pounds of potassium, and 16.08 pounds of nitrogen. The moisture was kept at 12.5, 15, 17.5, 20, and 22.5 per cent by weight. Duplicate pots were used in every case with each specific treatment. At the end of the experiment three separate analyses made on each pot, so that each reported result is the average of six closely agreeing determinations. The results are given in Table II.

The number of bacteria developing on synthetic agar does not seem to have been greatly influenced by the various treatments. All counts are comparatively low. If, however, we average the results for all pots which received the same manurial treatment we find a greater number developed from the soils which received 25 tons of manure to the acre than from any of the others. Moreover, there is an appreciable difference in favor of those soils receiving from 10 to 20 tons per acre over the unmanured soil. The irrigation water apparently depresses the number of organisms, for the greatest number developed from soil receiving the least water; but here also the difference is not marked or regular. The

average counts from the pots receiving the same quantities of irrigation water are with 12.5 per cent of water, 4,251,000; 15 per cent of water, 3,838,000; 17.5 per cent of water, 3,882,000; 20 per cent of water, 3,352,000; and 22.5 per cent of water, 3,680,000.

TABLE II.—*Number of bacteria developing on synthetic agar and quantity of ammonia and nitric nitrogen formed in 100 gm. of soil and of nitrogen fixed in 100 c. c. of Ashby solution—pot experiments*

Treatment.	Number of colonies of bacteria.	Quantity of ammonia formed.	Quantity of nitric nitrogen formed.	Quantity of nitrogen fixed.
		<i>Mgm.</i>	<i>Mgm.</i>	<i>Mgm.</i>
12.5 per cent of water; no manure. . . . .	3, 530, 000	36. 9	3. 36	9. 9
12.5 per cent of water; 5 tons of manure. . . . .	3, 300, 000	37. 9	5. 81	10. 3
12.5 per cent of water; 10 tons of manure. . . . .	4, 710, 000	48. 5	78. 05	10. 19
12.5 per cent of water; 15 tons of manure. . . . .	2, 810, 000	49. 1	88. 55	9. 94
12.5 per cent of water; 20 tons of manure. . . . .	6, 100, 000	49. 6	115. 00	10. 15
12.5 per cent of water; 25 tons of manure. . . . .	5, 060, 000	60. 5	110. 55	9. 73
15 per cent of water; no manure. . . . .	3, 360, 000	37. 6	4. 90	10. 19
15 per cent of water; 5 tons of manure. . . . .	3, 300, 000	43. 2	67. 90	10. 33
15 per cent of water; 10 tons of manure. . . . .	3, 660, 000	49. 0	84. 87	11. 50
15 per cent of water; 15 tons of manure. . . . .	4, 260, 000	51. 0	110. 55	11. 90
15 per cent of water; 20 tons of manure. . . . .	3, 720, 000	57. 1	113. 42	11. 02
15 per cent of water; 25 tons of manure. . . . .	4, 730, 000	74. 8	117. 25	10. 05
17.5 per cent water; no manure. . . . .	3, 800, 000	37. 4	3. 49	10. 57
17.5 per cent of water; 5 tons of manure. . . . .	3, 730, 000	48. 5	75. 60	10. 50
17.5 per cent of water; 10 tons of manure. . . . .	4, 330, 000	48. 6	88. 21	11. 46
17.5 per cent of water; 15 tons of manure. . . . .	4, 050, 000	54. 7	108. 85	9. 59
17.5 per cent of water; 20 tons of manure. . . . .	2, 920, 000	60. 9	111. 80	9. 69
17.5 per cent of water; 25 tons of manure. . . . .	4, 460, 000	69. 9	124. 40	9. 83
20 per cent of water; no manure. . . . .	3, 330, 000	38. 8	4. 20	9. 45
20 per cent of water; 5 tons of manure. . . . .	2, 860, 000	50. 0	73. 32	10. 36
20 per cent of water; 10 tons of manure. . . . .	4, 030, 000	54. 4	78. 14	10. 81
20 per cent of water; 15 tons of manure. . . . .	3, 430, 000	65. 4	106. 55	11. 20
20 per cent of water; 20 tons of manure. . . . .	3, 230, 000	69. 7	113. 00	10. 46
20 per cent of water; 25 tons of manure. . . . .	3, 230, 000	67. 9	125. 70	10. 60
22.5 per cent of water; no manure. . . . .	3, 730, 000	36. 2	7. 85	10. 54
22.5 per cent of water; 5 tons of manure. . . . .	4, 400, 000	48. 8	65. 74	10. 57
22.5 per cent of water; 10 tons of manure. . . . .	3, 030, 000	60. 8	81. 19	11. 83
22.5 per cent of water; 15 tons of manure. . . . .	3, 130, 000	63. 8	118. 50	10. 43
22.5 per cent of water; 20 tons of manure. . . . .	4, 530, 000	63. 1	119. 30	10. 95
22.5 per cent of water; 25 tons of manure. . . . .	3, 260, 000	64. 6	126. 65	10. 89

Both the water and the manure applied make a marked difference in the ammonifying powers of the soil. It is lowest in those pots which received no manure and gradually increases when 5, 10, 15, 20, and 25 tons of manure are applied. The water likewise has a noticeable effect on the ammonifying powers of the soil. In the unmanured soil it increases until 20 per cent of water is applied, at which point it reaches its maximum. When more than this quantity of water is applied, the ammonification is retarded. It is not as great when 22.5 per cent of water is applied as in the presence of only 12.5 per cent. Similar results are obtained when various quantities of water are applied in the presence of 5 tons of manure per acre. Here the influence of the water is much more pronounced than it is in the absence of manure. It reaches its maximum effect when 20 per cent of water is applied. In the presence of 10 tons per acre of manure the higher percentages of water have much greater influence on the ammonifying powers of the soil than do the lower percentages of water. In the presence of 20 tons of manure the water also exerts a great influence, but here the higher percentages produce a depressing effect, which becomes very perceptible in the pots which have received 25 tons of manure to the acre. It is interesting to note that with 25 tons of manure 15 per cent of water gave better results than either higher or lower percentages of water. It is quite possible that the higher water content in the presence of large quantities of organic matter produce anaerobic conditions which are not fully compatible with the best bacterial activities. The results are brought out more fully in figure 1, on the horizontal line of which is given the percentage of water applied to the soil, while on the perpendicular line is given the milligrams of ammonia produced in 100 gm. of soil.

If we consider the average quantity of ammonia produced in the unmanured pots as 100 per cent, that produced on the various manured pots becomes, with 5 tons of manure, 122 per cent; with 10 tons of manure, 140 per cent; with 15 tons, 152 per cent; with 20 tons, 160 per cent; and with 25 tons, 181 per cent. The average increase per ton of manure applied is greatest when 5 tons to the acre are applied and becomes gradually less as the quantity of manure applied becomes greater. If we consider the average percentage of ammonia produced in the soils receiving 12.5 per cent of water as 100, then the soil receiving 15 per cent of water produced 110 per cent; the soils with 17.5 per cent of water produced 111 per cent; the soils with 20 per cent of water, 123 per cent; and those receiving 22.5 per cent of water produced 119 per cent of ammonia—a gradual increase in the ammonia produced until the quantity of water applied exceeded 20 per cent.

The application of manure to a soil produces a very great increase in the nitrifying powers of the soil. The quantity of nitrates produced is very low in the soil receiving no manure but is greatly increased with the application of manure, even with so large a quantity as 25 tons per

acre. There is nothing in the results which would indicate denitrification in the presence of the largest quantities of organic matter applied in this experiment. The nitrifying powers of the soil increase with the water applied up to 17.5 per cent. Above this it has a slight depressing effect upon nitrification, probably caused by the production of an anerobic condition, but even with the highest percentage of water and 25 tons per acre of manure there is nothing in the results obtained which would indicate that denitrification had taken place. These results are brought out clearly in figure 2, in which is indicated on the horizontal line the per-

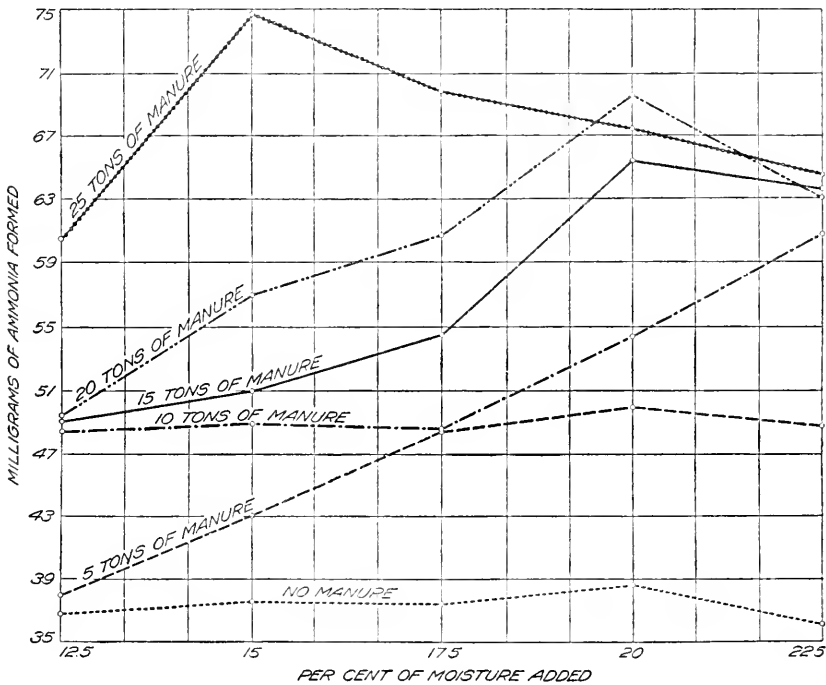


FIG. 1.—Curves of the ammonifying powers of soil in pots with varying quantities of manure and water.

centage of water and on the perpendicular line the milligrams of nitric nitrogen produced in 100 gm. of soil.

If we take the average of the nitric nitrogen produced in the unmanured pots as 100, then that of the manured pots becomes with 5 tons of manure, 1,211 per cent; with 10 tons, 1,762 per cent; 15 tons, 2,240 per cent; 20 tons, 2,405 per cent; and 25 tons, 2,540 per cent. The greatest increase per unit of manure is produced when 5 tons of manure are applied. The water applied also produces a gradual increase, but here likewise the greatest increase per unit of water applied is greatest for the lowest application of water.

The nitrogen-fixing powers of all the soils are fairly high, but the influence of the water and manure is not as pronounced as it is upon the

ammonifying and nitrifying powers of the soil. The results as a whole indicate that the manure increases the nitrogen-fixing power of the soil and it is slightly higher when 10 tons per acre of manure are applied to a soil than when any of the other quantities are applied. Even those

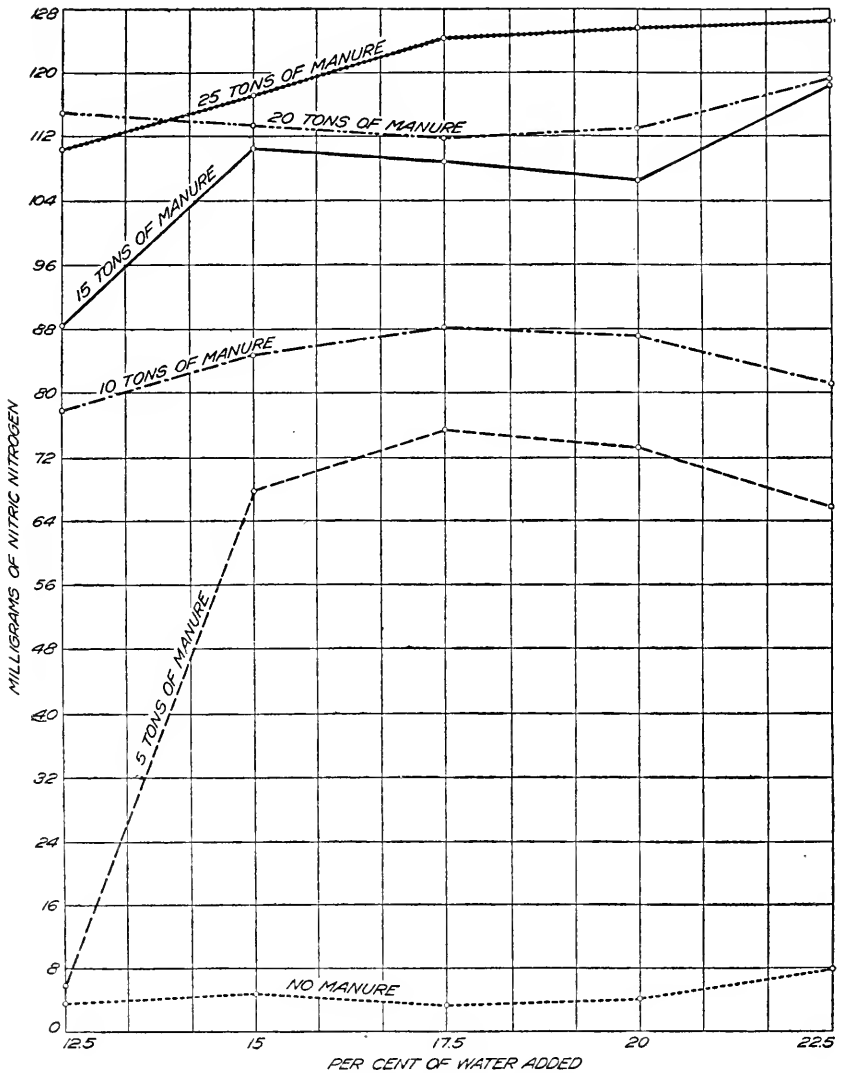


FIG. 2.—Curves of the nitrifying powers of soil in pots with varying quantities of manure and water.

pots receiving 5, 15, and 20 tons of manure per acre as an average fix more nitrogen than the unmanured soil.

The results taken as a whole indicate that the application of manure to soils in pot experiments influenced to a very great extent the ammonifying and nitrifying powers of a soil, but the influence upon the num-

ber of bacteria and nitrogen-fixing powers of the soil, while perceptible, is not as regular. The application of manure produced no difference in the temperature of the soil. The temperature of the manured and unmanured soils averaged very nearly the same throughout the experiment. The temperature of the pots receiving the least quantities of water averaged 1 degree centigrade higher than the soils receiving the greatest quantity of water.

The relationship existing in the various bacterial activities of the soil is brought out best by taking the average of each set of pots receiving the same quantity of manure and water. Then, if the bacterial activities of the pots receiving no manure and that of the pots receiving 12.5 per cent of water each be taken as 100 per cent and the others on a similar basis, we obtain a direct comparative value for each treatment. The results so obtained are given in Table III.

TABLE III.—*Bacterial activities of the soil in the presence of varying quantities of manure and water—pot experiments*

Treatment.	Bacteria.	Ammonia.	Nitric nitrogen.	Nitrogen fixed.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
No manure.....	100	100	100	100
5 tons of manure.....	99	122	1,211	103
10 tons of manure.....	111	140	1,762	110
15 tons of manure.....	100	152	2,240	105
20 tons of manure.....	116	160	2,405	103
25 tons of manure.....	117	180	2,540	101
12.5 per cent of water.....	100	100	100	100
15 per cent of water.....	90	111	118	108
17.5 per cent of water.....	91	113	121	102
20 per cent of water.....	79	123	121	104
22.5 per cent of water.....	87	119	123	108

It will be observed that the manure increases the number of bacteria developing upon the synthetic media, while the water depresses the number developing. In neither case is the regularity as great as could be desired. The ammonifying powers of the soil very regularly increases as the manure applied increases. The increase becomes less each time in a definite quantity as the manure increases. The water causes an increase in the ammonifying powers of the soil up until 20 per cent of water is applied; above this it causes a decrease. It would have been very interesting and practical to have added greater quantities of water to find whether it would have continued to depress the ammonification.

The quantity of nitric nitrogen systematically increases as the water and manure applied increase, and it may be seen, as would be expected, that there is a close correlation between the ammonification and nitrification. The nitrogen-fixing powers regularly increase up to 10 tons of manure per acre; above this they gradually decrease. The water tends

in all cases to increase the nitrogen gained. It will thus be observed that the manure applied increases the bacterial activities measured, while the water increased ammonification, nitrification, and nitrogen fixation, but depressed the number of colonies developing upon synthetic media. This would seem to be a very vital point against the count method. For we thus find a soil treatment increasing the main bacterial activities of a soil, but at the same time depressing the number developing in the laboratory. It would thus appear that the media used to make counts was better adapted for the development of organisms other than those which take the greatest part in the nitrogen transformation in the soil. On the other hand, it is quite possible that the increase in number may not keep pace with the increased physiological efficiency due to the application of water and manure. But this latter explanation would not account for the less number developing on the synthetic media.

#### FIELD EXPERIMENT ON FALLOW PLOTS

The fallow plots used in the field experiments were 7 feet wide and 24 feet long with a 4-foot walk between each two. The land was plowed in the fall, left over until spring, when a mixture of fairly well-rotted horse and cow manure was applied to the various manured plots. This was thoroughly disked or plowed into the soil. Water was applied to the plots from flumes as described in Utah Experiment Station Bulletins 115 to 120. They were kept free from weeds throughout the year. The quantities of water and manure applied to the various plots were as follows:

Four plots received no water and no manure.

Two plots received 5 inches of water, but no manure. The water was in two equal applications.

Two plots received 10 inches of water, but no manure. The water was applied in two equal applications.

Two plots received 20 inches of water, but no manure. The water was applied in four equal applications.

Two plots received 30 inches of water, but no manure. The water was applied in six equal applications.

Three plots received 40 inches of water, but no manure. The water was applied in eight equal applications.

All of the above were repeated with plots receiving 5 and 15 tons of manure to the acre. Hence, the series includes soils without manure, with 5 tons per acre, and with 15 tons per acre. The water applied varied from none up to 40 inches both with and without manure. This does not, however, represent the entire water reaching the soil, for there was an average annual precipitation of about 18 inches, most of which fell between the months of October and May. The precipitation from May to November did not exceed 5 inches, which, of course, would be



uniform for all plots. The plots had been treated since the spring of 1911 in the manner described; the bacteriological analyses were made during the summer of 1914 and 1915.

The results reported in Table IV giving the number of colonies of bacteria developing in four days on synthetic agar represent in every case the average of a number of determinations made at the times indicated.

TABLE IV.—*Number of colonies of bacteria developing in four days on synthetic agar—fallow plots*

Number of determinations.	Treatment.	Number of colonies.			
		May 12.	July 25.	Nov. 12.	Average.
12	No water; no manure...	3, 475, 000	12, 500, 000	4, 100, 000	5, 692, 000
6	5 inches of water; no manure.....	3, 000, 000	7, 600, 000	3, 700, 000	4, 767, 000
6	10 inches of water; no manure.....	2, 960, 000	12, 900, 000	3, 700, 000	6, 520, 000
6	20 inches of water; no manure.....	3, 030, 000	12, 600, 000	3, 950, 000	6, 527, 000
6	30 inches of water; no manure.....	2, 370, 000	15, 800, 000	5, 700, 000	7, 957, 000
9	40 inches of water; no manure.....	5, 660, 000	11, 860, 000	3, 800, 000	7, 107, 000
6	No water; 5 tons of manure.....	3, 570, 000	23, 500, 000	4, 300, 000	10, 457, 000
6	No water; 15 tons of manure.....	7, 700, 000	20, 000, 000	4, 800, 000	10, 833, 000
3	5 inches of water; 5 tons of manure.....	4, 000, 000	11, 800, 000	6, 600, 000	7, 467, 000
3	5 inches of water; 15 tons of manure.....	5, 600, 000	14, 200, 000	11, 200, 000	10, 333, 000
3	10 inches of water; 5 tons of manure.....	4, 600, 000	19, 000, 000	7, 600, 000	10, 400, 000
3	10 inches of water; 15 tons of manure.....	6, 000, 000	28, 000, 000	4, 000, 000	12, 667, 000
3	20 inches of water; 5 tons of manure.....	4, 300, 000	18, 000, 000	6, 600, 000	9, 633, 000
3	20 inches of water; 15 tons of manure.....	4, 400, 000	27, 400, 000	9, 800, 000	13, 867, 000
3	30 inches of water; 5 tons of manure.....	6, 200, 000	21, 200, 000	4, 400, 000	10, 600, 000
3	30 inches of water; 15 tons of manure.....	3, 600, 000	29, 400, 000	3, 200, 000	12, 067, 000
9	40 inches of water; 5 tons of manure.....	4, 450, 000	14, 066, 000	5, 933, 000	8, 150, 000
9	40 inches of water; 15 tons of manure.....	4, 600, 000	19, 933, 000	6, 200, 000	10, 244, 000

It may be seen that the number of organisms are comparatively low during the spring, in no case exceeding 8,000,000, while in July the number becomes in most cases three or four times as many. In November the number developing is about the same as in May. This method therefore gives a maximum count in midsummer. The spring samples were taken after all frost had left the ground, while the fall samples were taken before

there occurred any very hard frost; consequently these numbers do not in any case represent the numbers found in frozen soil, which would probably be higher than any of the results herein reported.

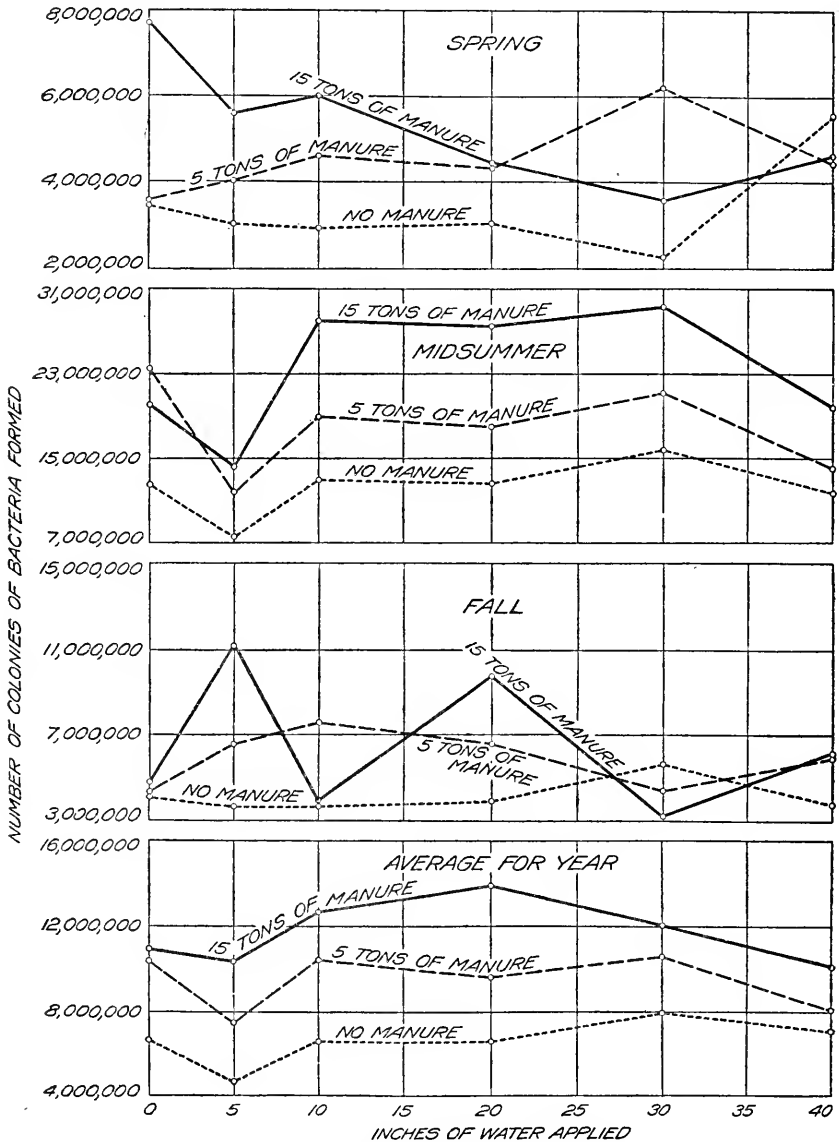


FIG. 3.—Curves of the number of colonies of bacteria developing from fallow soil with varying quantities of manure and water.

The results obtained for May show the unmanured soil to have few bacteria present, while the number in the manured soil increases as the quantity of manure increases. The water apparently had no marked effect upon their activity; or if it had, it had been obliterated during the winter

months. In July much the same order occurs. The soil receiving 15 tons of manure per acre contains more bacteria than that receiving 5 tons, and this in turn has more than the unmanured soil. Here the influence of the water becomes very marked, for there are many more bacteria in the soils receiving 10, 20, or 30 inches of water than in the soils receiving either no water or 40 inches. The excessive quantity of water, 40 inches, apparently checks the development of bacteria on the synthetic media.

The same results, in general, are obtained for November as for May and July, and with the exception of the abnormal results reported, where 10 inches of water were applied, the water has a pronounced effect even as late as November. This difference disappears during the winter, for we find a more uniform condition existing the next spring.

The average results for the unmanured soil show that more bacteria developed from the soil receiving 30 inches of water than from those receiving either more or less irrigation water. The manured soil, on the other hand, gave a maximum count from the soil receiving 20 inches of water. These differences are clearly brought out in figure 3. On the horizontal line is indicated the quantity of water applied, while on the perpendicular is given the number of colonies which developed. At the top of the figure are given the results for the spring, while below this in the order named for midsummer, fall, and the average for the year.

If we consider the average number of bacteria developing on synthetic media from the unmanured plots as 100 per cent, those developing on the manured plots become, with 5 tons of manure, 147 per cent, and with 15 tons, 177 per cent, showing that in so far as numbers are concerned the greatest effect per ton of manure applied is produced by the addition of 5 tons per acre. If we average the unirrigated plots and take these as 100 per cent, the others become, with 5 inches of water, 81 per cent; with 10 inches of water, 106 per cent; 20 inches of water, 107 per cent; 30 inches of water, 110 per cent; and 40 inches of water, 91 per cent. The maximum increase is apparently due to the application of 30 inches of irrigation water. But here, as was the case with the pot experiments, the results are not uniform.

The same plots were tested for ammonification, the results being given in Table V. In every case the result is the average of a number of closely agreeing determinations and are given as milligrams of ammonia produced in four days in 100 gm. of soil containing 2 gm. of dried blood.

The ammonifying powers of the soil, as may be seen from Table V, remain nearly constant throughout the season. There is, however, a big variation in the ammonifying powers of the different soils. In the spring the ammonifying powers of the unmanured soils are low. The quantity of ammonia formed in no case exceeds 57 mgm. per 100 gm. of soil. The water applied apparently had no perceptible influence upon the rate of ammonification. The quantity of ammonia produced by the soil receiving 5 tons per acre of manure is much higher than that pro-

duced by the unmanured, and the addition of water up to 10 inches produces a beneficial effect. The great effect, however, is noted on those soils which receive 15 tons of manure per acre. Here, also, the ammonifying powers are accelerated by the application of irrigation water up to 10 inches. Above this there is a depressing effect just as was noted in the pot experiments and can very likely be accounted for on the same grounds. In midsummer the influence of manure is just as perceptible as it is in the spring, and the influence of the water becomes much more regular, but still follows the same general trend that it did in the spring. In the fall the manure is found to exert almost quantitatively the same effect as it does in spring and midsummer. The depressing effect of the larger quantities of water during this season of the year is not as great as it is earlier in the year. But even here the higher applications (20 to 40 inches) cause a great falling off in the ammonifying powers of both the manured and unmanured soils. These results are brought out graphically in figure 4.

TABLE V.—Quantity of ammonia (in milligrams) produced in four days in 100 gm. of soil containing 2 gm. of dried blood—fallow plots

Number of determinations.	Treatment.	Quantity of ammonia.			
		May 12.	July 25.	Nov. 12.	Average.
12.....	No water; no manure.....	56.38	55.52	81.00	64.30
6.....	5 inches of water; no manure	54.78	47.60	77.10	59.82
6.....	10 inches of water; no manure.	50.99	46.25	64.65	53.96
6.....	20 inches of water; no manure.	49.56	44.65	62.80	52.33
6.....	30 inches of water; no manure.	46.62	44.30	61.70	50.87
9.....	40 inches of water; no manure.	49.87	42.83	63.97	52.22
6.....	No water; 5 tons of manure.	73.92	71.00	77.70	74.31
6.....	No water; 15 tons of manure.	92.65	82.95	82.25	85.95
3.....	5 inches of water; 5 tons of manure.	81.09	58.30	88.70	76.03
3.....	5 inches of water; 15 tons of manure.	116.55	97.60	92.00	102.05
3.....	10 inches of water; 5 tons of manure.	96.29	76.30	93.80	88.79
3.....	10 inches of water; 15 tons of manure.	129.20	118.50	112.00	119.90
3.....	20 inches of water; 5 tons of manure.	86.79	70.00	89.60	82.13
3.....	20 inches of water; 15 tons of manure.	111.59	108.5	106.6	108.89
3.....	30 inches of water; 5 tons of manure.	82.45	70.40	79.90	77.58
3.....	30 inches of water; 15 tons of manure.	112.63	.....	105.80	109.2
9.....	40 inches of water; 5 tons of manure.	98.78	79.47	90.50	89.58
9.....	40 inches of water; 15 tons of manure.	106.51	91.63	100.33	99.49

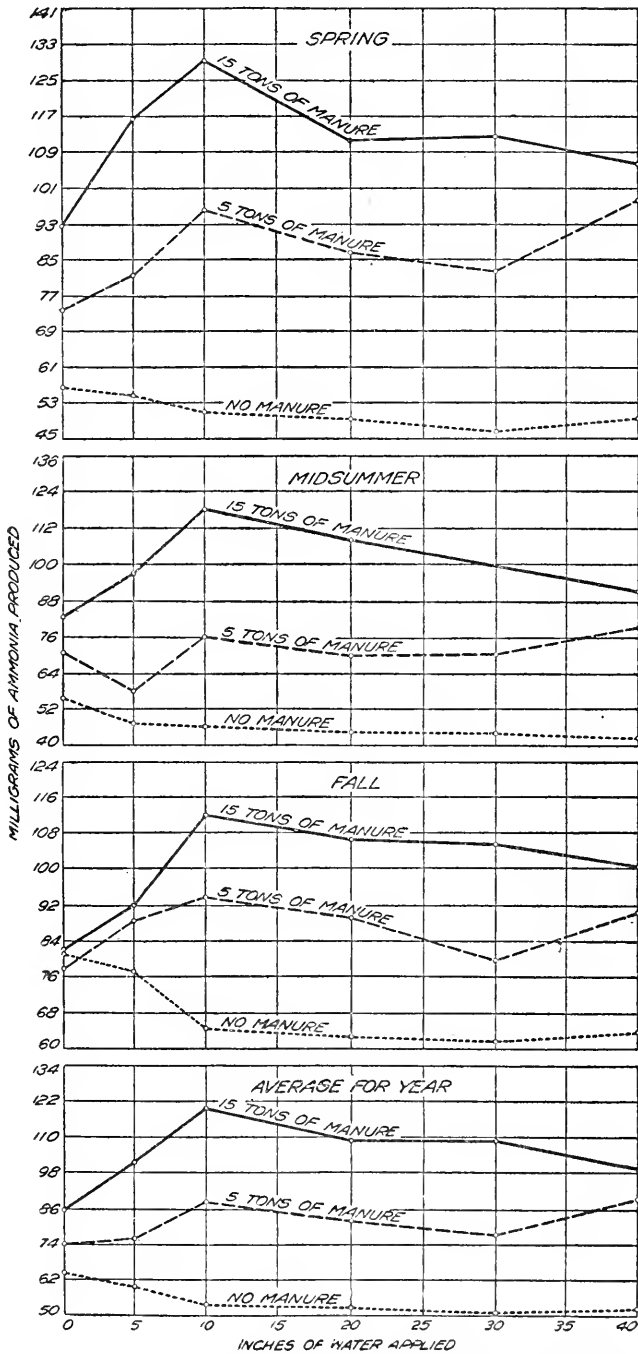


Fig. 4.—Curves of the ammonifying powers of fallow soil with varying quantities of manure and water.

If we take the average quantity of ammonia produced in the unmanured soil as 100 per cent and compare this with that produced in the manured soil, we find those soils receiving 5 tons of manure produce 147 per cent and those receiving 15 tons produce 188 per cent; or the average increase per ton of manure applied is twice as great when 5 tons are applied as when three times that much is used.

Considering the average of the soil receiving no irrigation water as 100 per cent, the others then become with 5 inches of water, 106 per cent; with 10 inches of water, 117 per cent; 20 inches of water, 108 per cent; 30 inches of water, 106 per cent; and 40 inches of water, 108 per cent. The greatest increase in ammonifying powers results from the application of 10 inches of irrigation water.

The nitrifying powers were determined as previously outlined, and the results reported in Table VI represent milligrams of nitric nitrogen formed during 21 days in 100 gm. of soil containing 2 gm. of dried blood. The results as reported are the average in each case of a number of determinations taken during two years.

TABLE VI.—Quantity of nitric nitrogen (in milligrams) produced in 21 days in 100 gm. of soil to which had been added 2 gm. of dried blood—fallow plots

Number of determinations.	Treatment.	Quantity of nitric nitrogen.			
		May 12.	July 25.	Nov. 12.	Average.
12	No water; no manure.....	1.46	16.36	2.16	6.66
6	5 inches water; no manure.....	1.40	11.90	.88	4.72
6	10 inches water; no manure.....	.79	13.85	.97	5.20
6	20 inches water; no manure.....	1.19	13.30	1.33	5.27
6	30 inches water; no manure.....	1.40	9.27	1.00	3.89
9	40 inches water; no manure.....	1.05	5.37	.89	2.43
6	No water; 5 tons of manure.....	1.47	10.32	2.15	4.64
6	No water; 15 tons of manure.....	11.90	40.43	30.50	27.61
3	5 inches water; 5 tons of manure.....	4.03	4.20	7.35	5.19
3	5 inches water; 15 tons of manure.....	1.75	45.20	31.85	26.27
3	10 inches water; 5 tons of manure.....	1.47	24.85	.70	9.01
3	10 inches water; 15 tons of manure.....	2.63	26.25	15.40	14.76
3	20 inches water; 5 tons of manure.....	1.23	7.70	11.20	6.71
3	20 inches water; 15 tons of manure.....	2.53	40.95	18.00	20.79
3	30 inches water; 5 tons of manure.....	.88	2.80	2.80	2.16
3	30 inches water; 15 tons of manure.....	2.52	21.00	46.90	23.47
9	40 inches water; 5 tons of manure.....	1.19	2.33	2.22	1.91
0	40 inches water; 15 tons of manure.....	2.63	25.78	33.65	20.69

All of these results will appear low when compared with those obtained by many other workers, who report their results as milligrams of nitrates found. The nitrifying powers of all the soils are low in the spring, but become much higher in midsummer and fall back in autumn to about where they were in the spring.

During the spring the nitrifying powers of the soil vary with the manure applied. But the difference existing between the manured and unmanured soil in no case is great. The irrigation water which had been

applied during the previous season exerted no effect which carried over the winter. In midsummer the nitrifying powers of the soil receiving 5 tons of manure are apparently less than the soil receiving no manure. The plots receiving 15 tons per acre are much more active in nitrifying dried blood than are the others. The lower applications of irrigation water apparently exert a favorable influence on all the plots, but the greater applications exert a depressing influence. It is, however, no more marked in the heavily manured soils than in the others; therefore, if there be any denitrification taking place, it must be attributed to the production of anaerobic conditions by the water, and not due to the manure applied. In November the beneficial influence of the 5 tons of manure applied becomes more regular than at any other time of the year. Here also the influence of the water becomes more perceptible. Taking the results as a whole they do not show the influence of either manure or water as well as it was shown by the potted soils; nor do they bring out the difference as clearly as it is brought out by the ammonification series. The relationship actually existing in the various treated soils is brought out graphically in figure 5.

On the base line is indicated the irrigation water applied in inches per acre, while on the perpendicular line is given the milligrams of nitric nitrogen produced in 100 gm. of soil to which 2 gm. of dried blood had been added. Taking the average nitric nitrogen produced in the unmanured soil as 100 per cent, the soil receiving 5 tons of manure becomes 105 per cent, while that of the soil receiving 15 tons becomes 486 per cent; or the average increase per unit of manure applied is much greater when 15 tons of manure are applied than when only 5 tons are applied. In this respect it differs markedly from the ammonification series.

Taking the average of the unirrigated plots as 100 per cent, the irrigated plots then arrange themselves in the order—5 inches, 94 per cent; 10 inches, 75 per cent; 20 inches, 85 per cent; 30 inches, 76 per cent; and 40 inches, 65 per cent. In every case the average for the season on all plots shows the water to have a depressing influence upon nitrification.

#### FIELD EXPERIMENTS ON CROPPED PLOTS

The same number of plots, arranged and treated exactly the same as those in the preceding part except that they were cropped, were sampled. These had grown corn continuously since the spring of 1911. They were sampled at the same time of the year, and bacterial counts made as was done on the fallow soil. The average results are given in Table VII.

These results are very similar to those obtained on the fallow soil. The number of organisms obtained is slightly lower and we do not find as great an increase during the summer months as we do on the fallow. The count as obtained in the spring is low for the unmanured soil, higher for that receiving 5 tons per acre of manure and still higher for the soil receiving 15 tons of manure. While the difference is marked,

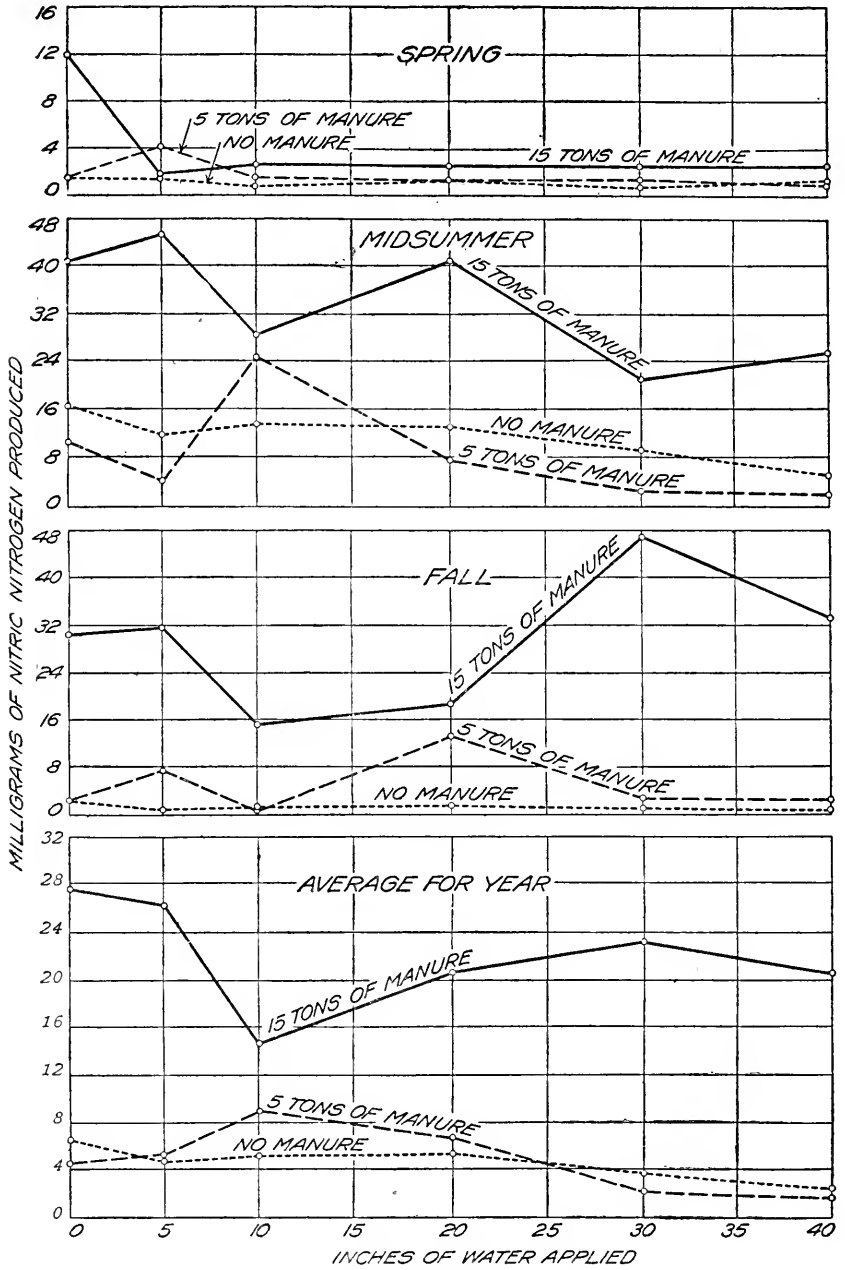


FIG. 5.—Curves of the nitrifying powers of fallow soil with varying quantities of manure and water.



it is not as pronounced as it is in the fallow soil. The same general order is seen during spring and fall, but in the fall the difference is greater in degree and more regular than in the earlier part of the year. The application of irrigation water produces an increase with the lower applications, especially on the heavily manured soil. The irregularity of this set as compared to the fallow can be accounted for in a degree by the error entering in sampling, for in some cases the sample may be taken nearer a plant than in others and in the cultivation and irrigation the tendency would be to leave the soil less homogeneous in the cropped than in the fallow plots. These conditions were borne in mind at the time of sampling and efforts made to get representative samples, but the results show that much more care must be taken on cropped than on fallow soil. The results for this series of plots are given graphically in figure 6.

TABLE VII.—*Number of colonies of bacteria developing in four days on synthetic agar—cropped plots*

Number of determinations.	Treatment.	Number of colonies.			
		May 10.	Aug. 9.	Nov. 8.	Average.
6	No water; no manure . . . . .	4, 300, 000	7, 300, 000	4, 000, 000	5, 200, 000
6	5 inches of water; no manure . . . . .	4, 500, 000	4, 250, 000	2, 700, 000	3, 817, 000
6	10 inches of water; no manure . . . . .	5, 800, 000	3, 950, 000	1, 800, 000	3, 850, 000
6	20 inches of water; no manure . . . . .	5, 200, 000	6, 150, 000	1, 800, 000	4, 387, 000
6	30 inches of water; no manure . . . . .	5, 100, 000	4, 600, 000	2, 000, 000	3, 900, 000
6	40 inches of water; no manure . . . . .	4, 300, 000	4, 700, 000	5, 700, 000	4, 900, 000
6	No water; 5 tons of manure . . . . .	8, 300, 000	4, 700, 000	3, 200, 000	5, 400, 000
6	No water; 15 tons of manure . . . . .	5, 300, 000	5, 400, 000	2, 200, 000	4, 300, 000
6	5 inches of water; 5 tons of manure . . . . .	6, 300, 000	5, 300, 000	2, 900, 000	4, 833, 000
6	5 inches of water 15 tons of manure . . . . .	8, 800, 000	6, 950, 000	6, 800, 000	7, 517, 000
6	10 inches of water; 5 tons of manure . . . . .	6, 100, 000	6, 300, 000	7, 800, 000	6, 733, 000
6	10 inches of water; 15 tons of manure . . . . .	6, 800, 000	5, 800, 000	4, 200, 000	4, 933, 000
6	20 inches of water; 5 tons of manure . . . . .	6, 100, 000	6, 350, 000	4, 500, 000	5, 633, 000
6	20 inches of water; 15 tons of manure . . . . .	5, 900, 000	5, 450, 000	4, 600, 000	5, 317, 000
6	30 inches of water; 5 tons of manure . . . . .	4, 200, 000	6, 900, 000	2, 800, 000	4, 633, 000
6	30 inches of water; 15 tons of manure . . . . .	5, 800, 000	6, 900, 000	3, 800, 000	5, 500, 000
6	40 inches of water; 5 tons of manure . . . . .	7, 600, 000	4, 450, 000	3, 500, 000	5, 183, 000
6	40 inches of water; 15 tons of manure . . . . .	5, 100, 000	7, 000, 000	7, 800, 000	6, 633, 000

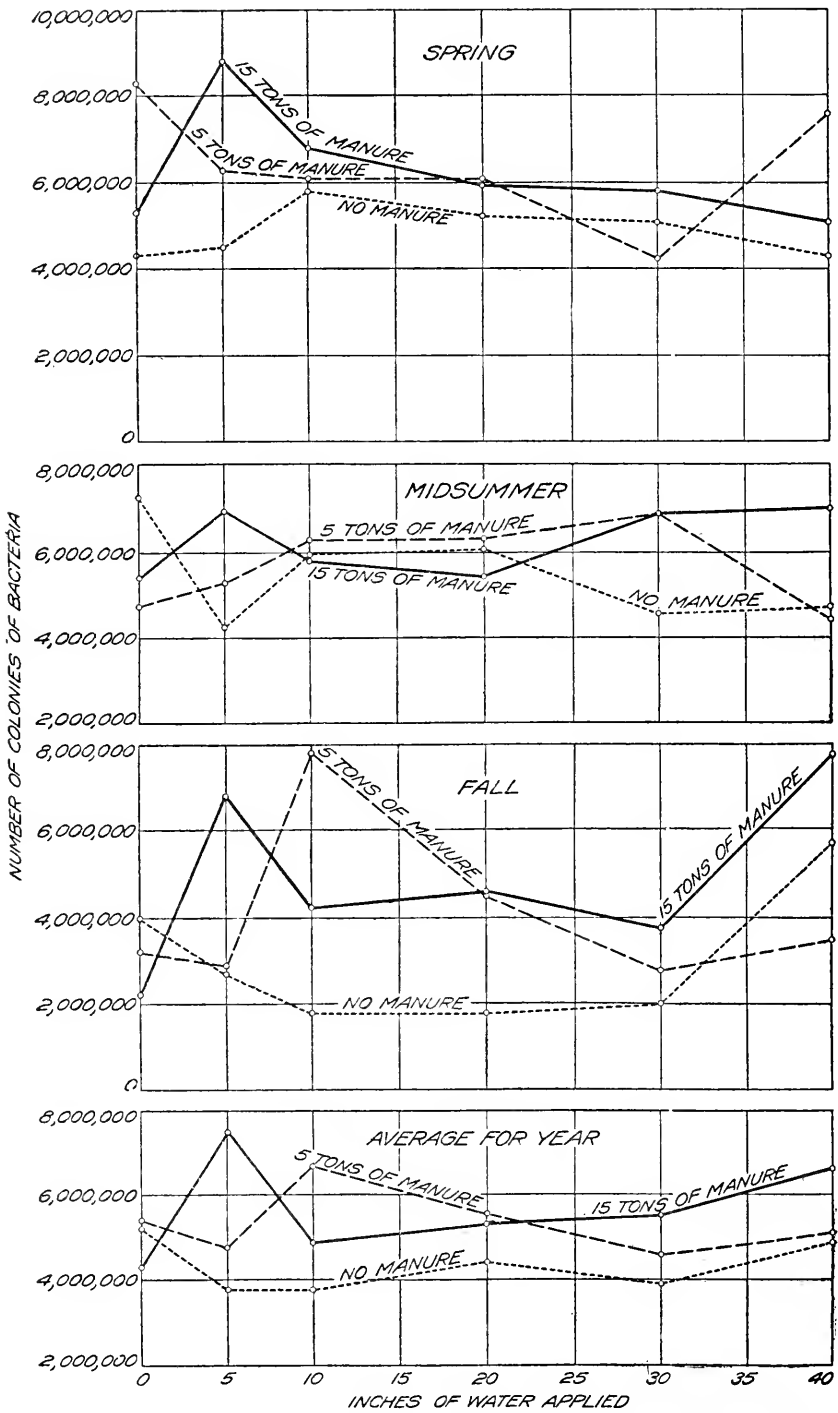


FIG. 6.—Curves of the number of colonies of bacteria developing from cropped plots with varying quantities of manure.

If the average number of bacteria found in the manured soil be taken as 100 per cent, the soil receiving 5 tons of manure then becomes 123 per cent and that receiving 15 tons, 129 per cent. Taking the average bacterial count of the plot receiving no irrigation water as 100 per cent, the others then become with 5 inches of water, 109 per cent; with 10 inches of water, 104 per cent; with 20 inches, 103 per cent; with 30 inches, 94 per cent; and with 40 inches, 112 per cent. With one exception the irrigation water had increased the number of bacteria in the soil.

The same plots were analyzed on the same dates for their ammonifying powers, and the results are given in Table VIII as milligrams of ammonia produced in four days in 100 gm. of soil, to which were added 2 gm. of dried blood. Each result is the average of a number of closely agreeing determinations.

TABLE VIII.—*Quantity of ammonia (in milligrams) formed in four days in 100 gm. of soil containing 2 gm. of dried blood—cropped plots*

Number of determinations.	Treatment.	Quantity of ammonia.			
		May 10.	Aug. 9.	Nov. 8.	Average.
6.....	No water; no manure.....	54.05	44.54	46.59	48.39
6.....	5 inches of water; no manure...	48.96	49.64	45.73	48.11
6.....	10 inches of water; no manure..	50.10	51.17	44.54	48.60
6.....	20 inches of water; no manure..	53.04	48.27	39.95	47.09
6.....	30 inches of water; no manure..	48.96	45.05	36.89	43.63
6.....	40 inches of water; no manure..	52.87	51.55	37.07	47.16
6.....	No water; 5 tons of manure.....	57.80	67.15	55.25	60.07
6.....	No water; 15 tons of manure....	71.69	67.15	68.85	69.23
6.....	5 inches of water; 5 tons of manure.....	60.33	60.69	53.17	58.06
6.....	5 inches of water; 15 tons of manure.....	91.63	74.41	73.79	79.94
6.....	10 inches of water; 5 tons of manure.....	61.08	70.69	61.54	64.44
6.....	10 inches of water; 15 tons of manure.....	92.99	89.93	87.05	89.99
6.....	20 inches of water; 5 tons of manure.....	63.16	61.54	56.10	60.26
6.....	20 inches of water; 15 tons of manure.....	96.69	101.15	89.45	95.76
6.....	30 inches of water; 5 tons of manure.....	57.97	68.17	51.34	59.16
6.....	30 inches of water; 15 tons of manure.....	97.41	115.20	76.16	96.26
6.....	40 inches of water; 5 tons of manure.....	63.07	67.49	51.01	60.52
6.....	40 inches of water; 15 tons of manure.....	86.87	91.63	77.20	85.23

The ammonifying powers of these soils are lower, as an average, in the cropped than in the fallow soil. The average quantity of ammonia produced by the fallow soil was 79.43 mgm., while that produced by the cropped soil was 64.48 mgm. The variation due to seasonal differences is not as great in the cropped as in the fallow soil, thus indicating that the influence of the season on the rate of ammonification is greatly offset

by crop and cultural methods. The variation between the differently treated soils during the same part of the year is qualitatively similar to that noted in the fallow soil.

The influence of the manure is very pronounced throughout the entire season. The ammonifying powers of the unmanured soils are all low, while those of soils receiving 5 tons of manure per acre are higher. Those of soils receiving 15 tons of manure per acre are very high. This difference is probably slightly greater during the spring months than during the fall.

The irrigation water applied is found to exert an influence upon this group of bacterial activities. Measured in terms of ammonification, the unmanured soils and those receiving 5 tons of manure per acre are benefited greatly by small quantities (10 and 20 inches) of irrigation water, while the soils receiving 15 tons of manure per acre have the highest ammonifying powers when they receive 20 or 30 inches of water. During the spring it is greatest in those soils from plots receiving 30 inches of irrigation water. Forty inches of water produce a marked depression in the ammonia formed, being pronounced in the soils receiving 15 tons of manure not only in the cropped soil but also in the fallow and potted soils. It is clear, therefore, that large quantities of water applied to a soil rich in organic matter depress the beneficial bacterial activities of that soil. The fallow unmanured soils and soils receiving 5 tons of manure per acre showed a slight decrease in the ammonifying powers of the soil, owing to the larger applications of irrigation water; but this does not appear in the cropped soil and is probably caused by the removal of large quantities of water by the growing crop, so that enough water does not accumulate in the presence of these small quantities of organic material to injure the ammonifying powers of the soil. These facts are brought out clearly in figure 7.

If we take the average of the quantity of ammonia produced in the unmanured soil as 100 per cent, the others then become with 5 tons 129 per cent and with 15 tons 183 per cent. Here the average increase per ton of manure applied is about the same whether 5 or 15 tons of manure be applied per acre. If the average of the plots receiving no irrigation water be taken as 100 per cent, the others then become with 5 inches of water 105 per cent; with 10 inches, 114 per cent; 20 inches, 118 per cent; 30 inches, 112 per cent; and 40 inches, 109 per cent. It thus reaches its maximum when 20 inches of water are applied, while the fallow reached its maximum when only 10 inches were applied. This is a difference which is undoubtedly due to the great quantities of water removed by the growing plant. The average increase per acre-inch of water, however, is greatest in the cropped soil where only 10 inches of irrigation water were applied.

The nitrifying powers of the same soils were tested by the method previously given, the results of such tests being given in Table IX as milligrams of nitric nitrogen produced during 21 days in 100 gm. of

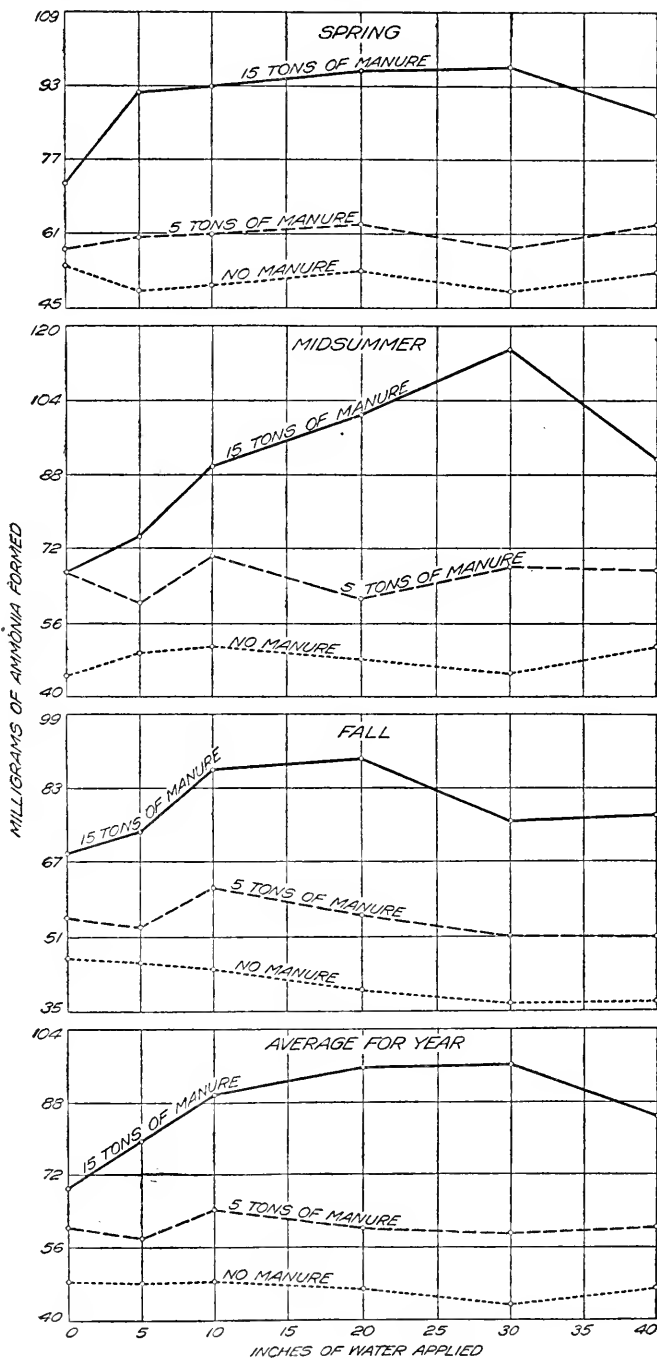


FIG. 7.—Curves of the ammonifying powers of soil of cropped plots with varying quantities of manure and water.

soil containing 2 gm. of dried blood. All the reported results are the average of two or more closely agreeing determinations.

TABLE IX.—Quantity of nitric nitrogen (in milligrams) formed in 100 gm. of soil containing 2 gm. of dried blood

Number of determinations.	Treatment.	Quantity of nitric nitrogen.			
		May 10.	Aug. 9.	Nov. 8.	Average.
6.....	No water; no manure.....	1. 50	1. 33	0. 84	1. 22
6.....	5 inches of water; no manure....	3. 47	. 81	. 88	1. 72
6.....	10 inches of water; no manure..	2. 27	. 56	. 53	1. 12
6.....	20 inches of water; no manure..	1. 05	. 50	1. 85	1. 13
6.....	30 inches of water; no manure..	1. 40	. 38	. 35	. 71
6.....	40 inches of water; no manure..	2. 10	. 70	1. 15	1. 31
6.....	No water; 5 tons of manure.....	1. 65	1. 80	2. 45	1. 97
6.....	No water; 15 tons of manure....	45. 32	8. 85	27. 52	27. 23
6.....	5 inches of water; 5 tons of manure.....	20. 30	. 53	3. 43	8. 08
6.....	5 inches of water; 15 tons of manure.....	46. 80	2. 66	33. 65	27. 70
6.....	10 inches of water; 5 tons of manure.....	4. 90	. 58	8. 48	4. 65
6.....	10 inches of water; 15 tons of manure.....	47. 45	2. 27	22. 92	24. 21
6.....	20 inches of water; 5 tons of manure.....	9. 27	. 63	2. 66	4. 18
6.....	20 inches of water; 15 tons of manure.....	53. 05	2. 17	23. 80	26. 34
6.....	30 inches of water; 5 tons of manure.....	12. 30	. 40	2. 80	5. 17
6.....	30 inches of water; 15 tons of manure.....	60. 25	15. 46	4. 93	26. 88
6.....	40 inches of water; 5 tons of manure.....	18. 37	. 45	6. 75	8. 52
6.....	40 inches of water; 15 tons of manure.....	37. 05	1. 01	14. 08	17. 38

The nitrifying powers of these soils are uniformly higher in the spring months of the year than later. This occurs in all the plots, but the greatest difference is found in the heavily manured plots, due probably to the application of large quantities of readily nitrifiable material in the manure, which is transformed later into soluble nitrates taken up by the growing plant, removed in the drainage water, or transformed into complex protein substances within the bodies of various microorganisms. The results taken as a whole bear a very great similarity to those obtained on the fallow soil. They are, however, as were the counts and ammonifying powers, slightly higher in the fallow than in the cropped soil.

The nitrifying powers of the unmanured soil are low throughout the year. The nitrates produced by the manured soil increase with the increase of manure applied. The greatest difference, however, exists between the soil receiving 5 and 15 tons of manure per year. In the latter the nitrifying activity is extremely active in the spring months. This difference, while not as pronounced later in the year, exists throughout the season.

The irrigation water exerts a great influence upon the nitrifying powers of the soil and this follows almost exactly the order followed by the ammonifying series. It is greatest when a medium amount of water is applied, but becomes injurious as greater quantities of water are applied to the soil, especially with large quantities of organic matter. One could not conclude from these results that the quantities of water here applied in the presence of organic manure favor denitrification, but it is certain that the conditions thus produced are not the best for the nitrate and ammonia-forming organisms, and it is quite likely due to the anerobic condition produced by the excess of water. It is interesting to note that larger quantities of water are required on a cropped soil to exert this depressing influence than on a fallow soil. The results for this series are given graphically in figure 8.

Taking the average quantity of nitric nitrogen produced in the unmanured soil as 100 per cent, the soil receiving 5 tons of manure then becomes 453 per cent, while the percentage produced in the soils receiving 15 tons per acre becomes 2,079. Thus, an enormous increase is due directly to the application of manure to the soil.

Taking the average quantity of nitric nitrogen produced in the soil receiving no irrigation water as 100 per cent, the irrigated soils produced with 5 inches of water, 126 per cent; with 10 inches of water, 99 per cent; 20 inches of water, 104 per cent; 30 inches of water, 108 per cent; and 40 inches of water, 89 per cent—an unmistakable reduction in the nitrifying powers of soils receiving 40 inches of irrigation water.

RELATIONSHIP IN BACTERIAL ACTIVITIES IN POTTED, CROPPED, AND UNCROPPED SOIL

If we use in every case the quantity of ammonia and nitric nitrogen produced and the total number of bacteria developing from the unmanured in the one case and the unirrigated in the other as 100 per cent, we have a direct comparison between the bacterial activities of the variously treated soils. The results so obtained are given in Table X.

TABLE X.—*Comparison of the bacterial activities in the potted, fallow, and cropped soils*

Treatment.	Bacteria.			Ammonia.			Nitric nitrogen.		
	Pots.	Fallow.	Cropped.	Pots.	Fallow.	Cropped.	Pots.	Fallow.	Cropped.
No manure. . . . .	<i>Per ct.</i> 100	<i>Per ct.</i> 100	<i>Per ct.</i> 100	<i>Per ct.</i> 100	<i>Per ct.</i> 100	<i>Per ct.</i> 100	<i>Per ct.</i> 100	<i>Per ct.</i> 100	<i>Per ct.</i> 100
5 tons of manure. . . . .	99	144	123	122	147	129	1,211	105	453
15 tons of manure. . . . .	100	177	129	152	188	183	2,240	486	2,079
No irrigation water. . . . .	<i>a</i> 100	100	100	<i>a</i> 100	100	100	<i>a</i> 100	100	100
5 inches of water. . . . .	<i>b</i> 90	81	109	<i>b</i> 111	106	105	<i>b</i> 118	94	126
10 inches of water. . . . .	<i>c</i> 91	106	104	<i>c</i> 113	117	114	<i>c</i> 121	75	99
20 inches of water. . . . .	<i>d</i> 79	107	103	<i>d</i> 123	108	118	<i>d</i> 121	85	104
30 inches of water. . . . .	<i>e</i> 87	110	94	<i>e</i> 119	106	112	<i>e</i> 123	76	108
40 inches of water. . . . .	.....	91	112	.....	107	108	.....	65	89

*a* 12.5 per cent applied.  
*b* 15 per cent applied.

*c* 17.5 per cent applied.  
*d* 20 per cent applied.

*e* 22.5 per cent applied.

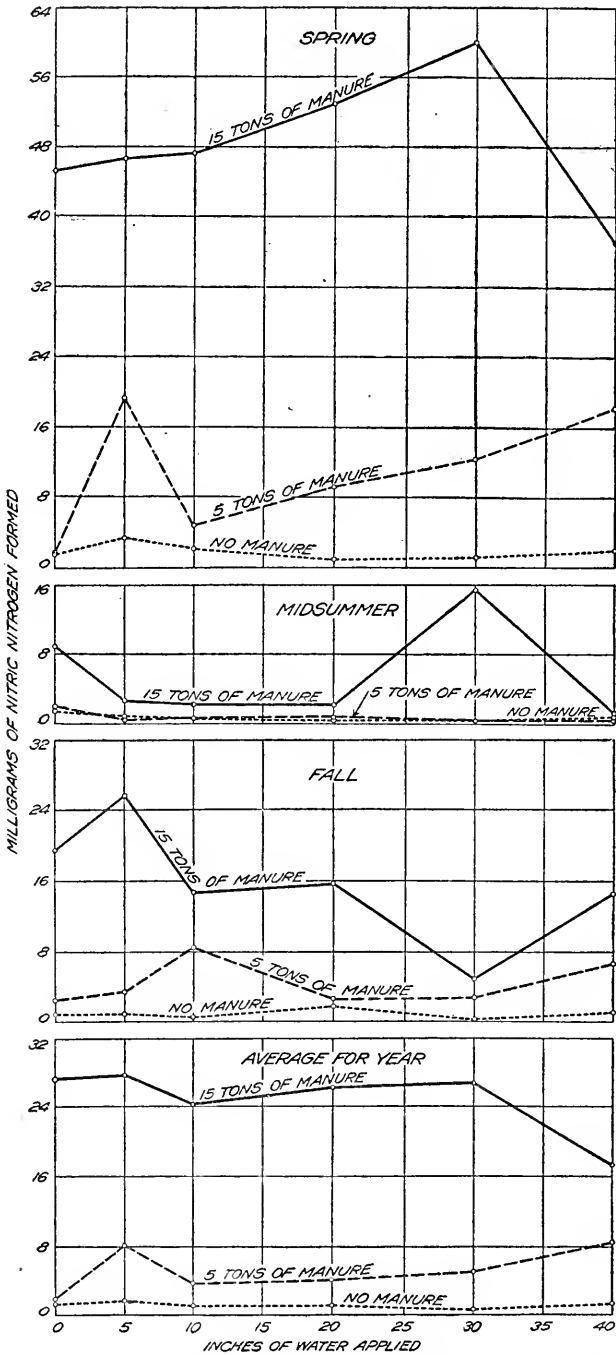


FIG. 8.—Curves of the nitrifying powers of soil of cropped plots with varying quantities of manure and water.



The results for manure show a remarkable uniformity throughout. With one exception it has increased the bacterial count and also the bacterial activities of the soil, and this is about the order throughout. The ammonifying and bacterial counts are increased more by the manure in the fallow than in the cropped soil.

The irrigation water applied apparently increases the bacterial count in the fallow and cropped field soil but it apparently depresses it in the potted soil. The ammonifying powers of all soils are uniformly increased with increasing amounts of irrigation water applied up to a certain application. Above this there is a depression. Greater quantities of water must be applied to cropped than uncropped soil in order to cause this depression. This is mainly owing to the influence of the plant upon the moisture content of the soil.

The nitrifying powers of the potted soils are very uniform in showing a beneficial effect due to the water. The cropped soil is not so uniform, while the fallow soil shows a depressing influence. These apparently contradictory results are quite likely caused by a difference in treatment, for the water in the three different sets of soil may have been far from the same.

#### RELATIONSHIPS BETWEEN BACTERIAL ACTIVITIES AND CROP-PRODUCING POWERS

The results herein reported, together with those published by Dr. Harris (19) upon Greenville soil, make it possible to compare directly the crop produced on the soil as an average of five years with the bacterial activities of the soil. This is done in figures 9 and 10, in which the bacterial activities and crop-producing powers of the unmanured soil are taken as 100 per cent and each of the manured plots compared with this. In the case of water applied the bacterial activities and crop produced upon the soils receiving no irrigation water are taken as 100 per cent and the others compared with this.

An examination of figure 9 shows a remarkably close correlation between the crop produced and the bacterial activities of the soil. The extent to which the bacterial count and ammonifying powers of the soil are increased by the manure applied is almost quantitatively the same as the increase in the crop produced on the manured soil. The increase in the nitrifying powers of the soil is much greater than the crop increase due to manure, but they are all of the same order.

An examination of figure 10 reveals the fact that the application of 5 inches of irrigation water increases in nearly the same proportion the crop produced and the bacterial activities of the soil. The average percentage for the crop is 112, while the total average bacterial activities is 113 per cent. The crop produced on the soil receiving 10 inches of water is slightly less than that produced on the soil receiving 5 inches

of irrigation water. With the exception of the ammonia produced, the bacterial activities are not as high in the soil receiving 10 as in the soil receiving only 5 inches of irrigation water. The average percentage of the crop produced on this is 110, while the average of the bacterial activities is 102 per cent. The application of 20 inches of irrigation water greatly increased the crop produced and also the bacterial activities, the crop produced being 127 per cent compared with the unirrigated, while using the same comparison for bacterial activities gives 108 per cent. The application of 30 inches of irrigation water causes a slight decrease in the corn produced and also in the bacterial activities of the soil, 40 inches of irrigation water producing about the same crop as did 30 inches. But it caused a slight falling off in the bacterial activities of the soil, especially in the nitrifying powers of the soil. Taking the result as a whole, we find that the bacterial activities of the soil and the crop-producing powers of a soil are both influenced by the application of irrigation water and this in the same direction and in about the same degree. These results tend to indicate that the bacteriological

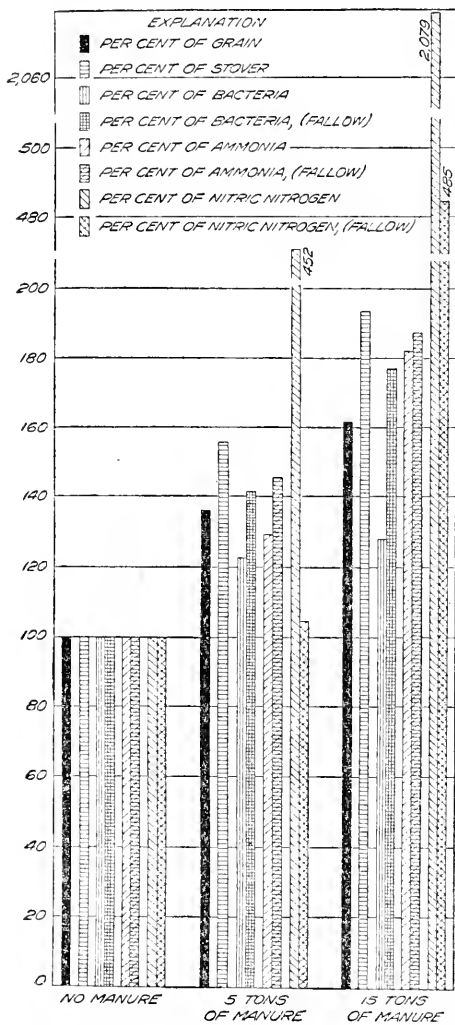


FIG. 9.—Diagram of the influence of manure on the yield and bacterial activities of a soil, the unmanured plots being expressed as 100 per cent.

analysis of a soil gives a fair insight into its relative crop-producing powers, being especially true with regards to the ammonifying and nitrifying powers of the soil.

#### SUMMARY

A calcareous soil kept in pots with varying amounts of manure and different percentage of moisture gave on bacteriological analyses at the end of four months the following results.

The temperature of the manured and unmanured averaged practically the same for the period, but the temperature of the soil with 12.5 per cent of water averaged 1 degree centigrade higher than did soils with 22.5 per cent of water. The greatest number of organisms developed on synthetic media from the soils receiving the greatest quantity, 25 tons, of manure. There were more colonies developed from the soil receiving 12.5 per cent of water than from any of the other soils receiving higher quantities of water.

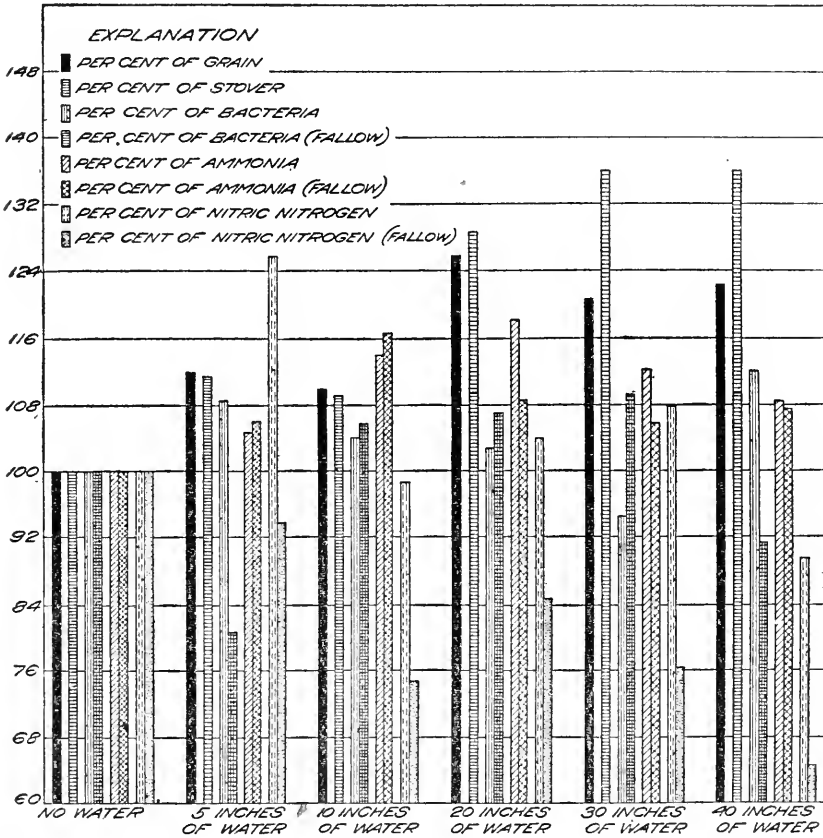


FIG. 10.—Diagram of the influence of irrigation water on the yield and bacterial activities of a soil, the nonirrigated plots being expressed as 100 per cent.

The ammonifying powers of the soil increased with the manure applied up to 25 tons of manure per acre, but the greatest increase per ton of manure was obtained in soil receiving 5 tons.

The ammonifying powers of the soils increased as the water applied increased until 20 per cent of water was applied. The ammonifying powers of soil receiving 22.5 per cent of water were not as high as were those of soil receiving 20 per cent of water. The greatest increase per unit of water applied was when the water was increased from 12.5 to 15 per cent of water.

The nitrifying powers of the soil increased as the manure and water applied increased up to 25 tons of manure and 22.5 per cent of water.

The nitrogen-fixing powers of the soil were greatest in those pots receiving at the rate of 10 tons of manure per acre. Increasing the water above 12.5 per cent but not above 22.5 per cent slightly increased the nitrogen-fixing powers of the soil. Nothing in the results indicated that the application of manure up to 25 tons per acre and of water up to 22.5 per cent caused denitrification in the soil.

Bacteriological analyses of fallow field soil receiving none, 5 tons, and 15 tons of manure per acre and receiving none, 5 inches, 10 inches, 20 inches, 30 inches, and 40 inches of irrigation water gave the following results.

The maximum number of bacteria were obtained from the soil receiving 15 tons of manure. The application of irrigation water up to 20 inches increased the bacterial count, being most noticeable in the soil receiving the greatest quantity of manure.

If the ammonifying powers of the unmanured soils are considered as 100 per cent and the unirrigated as 100 per cent, the manured and irrigated soils then become with 5 tons of manure, 147 per cent; with 15 tons of manure, 188 per cent; 5 inches of water, 106 per cent; 10 inches of water, 117 per cent; 20 inches of water, 108 per cent; 30 inches of water, 106 per cent; and 40 inches of water, 108 per cent. Large quantities of irrigation water produced the greatest depressing effect in the presence of 15 tons of manure per acre.

The application of manure to a soil increases its nitrifying powers. The application of irrigation water to a fallow soil apparently depresses its nitrifying powers.

Fewer organisms develop on synthetic agar from a cropped than from a fallow soil. The application of manure to a cropped soil increases the bacterial count of the soil. The greatest number of organisms developed from the soil receiving 10 inches of irrigation water.

The ammonifying powers of the cropped soils were slightly lower than similarly treated fallow soils. The application of 5 and 15 tons of manure per acre to a soil increases the ammonifying powers of the soil. The application of irrigation water up to 30 inches increases the ammonifying powers of the soil. The greatest increase resulted in those soils receiving 15 tons per acre of manure. The application of 40 inches of irrigation water to corn land, especially to that receiving 15 tons of manure per acre, depresses the ammonifying powers of the soil.

The nitrifying powers of fallow soil were higher than similarly treated cropped soils. The application of manure to a cropped soil greatly increases its nitrifying power. The application of irrigation water up to 30 inches, especially to a soil receiving 15 tons of manure per acre, greatly increases its nitrifying powers.

There was found to be a direct relationship between the bacterial count, the ammonifying powers, the nitrifying powers, and the crop produced on a soil receiving no manure, 5 tons, and 15 tons of manure per acre.

A close correlation was also found to exist between the bacterial activities of soil receiving varying amounts of water and crop produced upon the soil.

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## PROGRESSIVE OXIDATION OF COLD-STORAGE BUTTER

By D. C. DYER,

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### OUTLINE OF PREVIOUS WORK

Much has been written concerning the changes occurring in butter. The word "change" is here used in its broad and general sense to include any perceptible alteration whatsoever, although it refers principally to an organoleptic one, whether induced by one or several factors.

Butter has been kept for certain periods of time during which it has been exposed to the action of various decomposing and disintegrating agencies, and a study of the products of change thereby resulting has led investigators to draw conclusions relative to the causation of the "off flavors" so often found in stored butter. As a general rule, the majority of opinions advanced in accounting for the deterioration of butter seem to have been based either upon insufficient analytical data or upon a study of butter or butter fat kept under conditions which prevail only to a very limited degree when butter is stored.

Many investigators confined their attention to a study of the fat of butter alone and sought to attribute the appearance of undesirable flavors in whole butter to some change which this one constituent undergoes. However, more recent investigations carried on with fats other than butter would appear to render such an assumption doubtful and would seem to make imperative more conclusive information concerning the causation of disagreeable flavors in whole butter held in cold storage.

The early literature in regard to the chemical changes which take place in butter is voluminous, but it is also conflicting and confusing, a great deal of it being of a purely speculative nature.

A great variety of bodies, products of chemical change, have presumably been identified in butter kept under varying conditions. The confirmation of the presence after a certain interval of time of such substances in fats known to have been originally pure is of value; yet such data obtained in the investigation of a material containing other constituents as well are obviously not so satisfactory unless it is definitely known that these attendant components do not likewise undergo

similar changes. Acids, aldehydes, alcohols, and esters, among other things, may have been identified in spoiled fats, and even up to the present time it has been customary to attribute their origin solely to the fat itself. The reason for such deduction is evident. It is well known that the fat of butter is in itself a most complex material. It is a composite, made up of mixtures of the glycerids of fatty acids. Among the saturated glycerids butyric is an essential ingredient, although palmitic and myristic predominate. Oleic has generally been considered to be the only unsaturated glycerid in butter fat, yet quite recently Laxa and Konecny (3)<sup>1</sup> claim to have found that the fatty acids of the "liquid fat" of separator slime consist of 49.65 per cent of erucic acid and 21.24 per cent of oleic acids; but this assumption may not be entirely justified.

The improbability of any chemical change occurring in the saturated glycerids of storage butter is quite generally recognized; consequently the glycerid oleic, purely because it contains an unsaturated linkage in the molecule and absorbs the halogens with avidity, has been considered as the source from which are derived those decomposition products the presence of which in fats influences their more or less decreased value. As a matter of fact, any satisfactory and conclusive evidence that the oleic of butter fat is readily susceptible to oxidation under conditions similar to those prevailing when butter is stored is entirely lacking. On the other hand, it has been demonstrated that pure olive oil, the liquid glycerids of which consist almost entirely of oleic, shows very little absorption of oxygen as measured by the iodine number, even after having been kept for three years under ordinary conditions (5). Masters and Smith (6), in preliminary experiments with butter fat, found but little change in the iodine value during cooking experiments carried out with this material. To obtain any pronounced change in the iodine value and in the acidity, they found it necessary to heat their samples of butter fat to as high a temperature as 200° C. while passing oxygen through the material, the mere heating of the fat to such temperature under ordinary conditions proving to be insufficient. From these two illustrations, as well as from more recent work done by other investigators, the discussion of which owing to limited space is omitted, it must be concluded that the possibility of the oleic of butter fat undergoing an appreciable oxidation caused by the small quantity of atmospheric air inclosed in a package of butter is very remote, especially when it is remembered that butter is stored in the dark at a temperature considerably lower than the freezing point of water.

The inability of chemists to judge the quality of an edible fat because of the absence of satisfactory chemical data has been frequently pointed out, and this is attributable primarily to the lack of appropriate and comprehensive analytical procedure. For instance, rancidity has generally been regarded as the natural concomitant of acidity, yet a pronounced

<sup>1</sup> Reference is made by number to "Literature cited," p. 931.

rancidity may have appeared in a stored fat without the manifestation of any increased acidity as measured by a simple titration. Again, an undue significance may be attached to a slight decrease in the original iodine number of the fat. Such a decrease is usually considered to be caused by the taking up of oxygen by the double bond of the unsaturated glycerid; yet it must be remembered that self-polymerization—the interlocking of two or more molecules of the unsaturated glycerids—may occur, a condition which would likewise bring about a lowering of the iodine value. Again, the olein of butter fat may not exist entirely as the normal glycerid, and it is possible that a certain amount of this glycerid may occur as an isomerid. So far as is known to the writer no work has been carried out to determine whether the olein of stored butter is present entirely as the normal glycerid. In this connection it may be observed that the work of Ponzio and Gastaldi (8) and of Fokina (1) indicates that the farther the double bond is removed from the carboxyl group the nearer the iodine number approaches the theoretical value. Normal oleic acid gave the theoretical value of 90. On the contrary, 2-3 oleic acid gave a Hübl number of only 6.6, Wijs 20.4, Hanus 1.9. While there are no data at hand at present to prove that 2-3 oleic acid actually does occur in butter fat, yet this contingency is quite possible; and it is well to take it into consideration as yet another factor which may produce a slight lowering of the iodine number of stored butter. It is evident, however, that the customary methods in vogue to determine the quality of fat leave much to be desired.

One of the factors so often construed as influencing the appearance of undesirable flavors in a fat is the nature of the impurity, or impurities, contained therein. In just what manner these foreign substances bring about these undesirable characteristics has not been fully cleared up, because it is conceivable that it depends upon several parallelly progressing chemical reactions and because it is possible that slight chemical changes really difficult of identification by analytical methods suffice to produce the above-mentioned disagreeable features.

It is apparent that even at the present time there seems to be considerable doubt as to whether the undesirable flavors of storage butter arise from a decomposition occurring in the fat itself or in some one or more of the other components entering into the composition of the whole product. For this reason it is thought advisable to confine the preliminary work on this subject to an attempt to settle this most basic consideration before proceeding with the further investigation of the causation of the "off flavors" so frequently met with in storage butter.

#### STATEMENT OF THE PROBLEM AND METHOD OF SOLUTION

Even in those times when the chemical constitution of the fats was still unknown it had been surmised that the changes which oils and fats underwent on keeping were simply the result of oxidation. This is the

view most generally held at the present time, and the more recent literature on the subject indicates that this phase of research is to be continued with no less abated interest. It is still unknown whether the development of undesirable flavors in storage butter is dependent upon an oxidation occurring in the fat itself or whether the milk sugar and nitrogenous constituents of the curd are those components of the butter most susceptible to oxidation. Approximately 10 per cent of the volume of butter is air (9), and it is quite possible that, owing to the oxygen of the air inclosed within the material, a slight and progressive oxidation may take place in the interior of a package of butter. This possibility, when considered together with the known fact that marked and undesirable alterations in the flavor of butter during storage may be brought about by acidifying the pasteurized cream from which the butter is made (10), has suggested the idea that an examination of the air inclosed within packages of butter differently prepared and in butter fat alone might furnish some interesting data as to whether the undesirable chemical changes occurring in stored butter are caused by a progressive oxidation in the fat itself or in some one or more of the nonfatty ingredients.

It was deemed advisable to pursue this line of investigation in a manner not previously attempted, so far as known. Samples of pasteurized sweet-cream butter, butter made from pasteurized cream to which lactic acid had been added, and butter made from pasteurized cream to which a starter had been added and which was churned at once, were prepared, packed in glass tubes, and stored. Tubes from each lot were removed from storage after certain intervals of time had elapsed and an analysis of the air therefrom was made by means of the gas apparatus specially designed for the purpose. (See fig. 1 and Pl. CXI.) It was hoped that the analytical data so obtained would show some distinguishing features between the three samples dissimilarly prepared, especially with respect to the sample made from acid cream. It was also decided to make use of the determination of the chemical constants of the pure butter fat to serve merely as an indication as to whether any chemical alteration of the fat through oxidation had occurred during the storage interval, confirmed by the analysis of the air extracted from packages of butter fat to determine whether the oxygen content therein is diminished during the storage period. The data so obtained were used as a standard, and the aim kept in view was to study the effect, if any, of the presence of varying amounts of nonfatty constituents (protein, lactose, etc.) upon the decomposition of the fat of butter and, in addition, to note whether the presence of varying quantities of these substances in the butter induced an alteration during storage in the composition of the air incorporated in the samples at the time of their manufacture. Samples of pure butter fat and of butter containing varying quantities of buttermilk

were also prepared, packed into tubes, and stored under the same conditions as the foregoing samples. The effect of a large amount of air upon a small quantity of butter fat and upon buttermilk containing varying quantities of acid was studied by filling other tubes with pumice fragments which were then impregnated with fat or buttermilk and an analysis of the air therefrom made after certain intervals in storage had elapsed.

DESCRIPTION AND MANIPULATION OF THE GAS APPARATUS USED

In figure 1 is depicted the apparatus constructed for use in the extraction and analysis of the air confined in the packages of the various

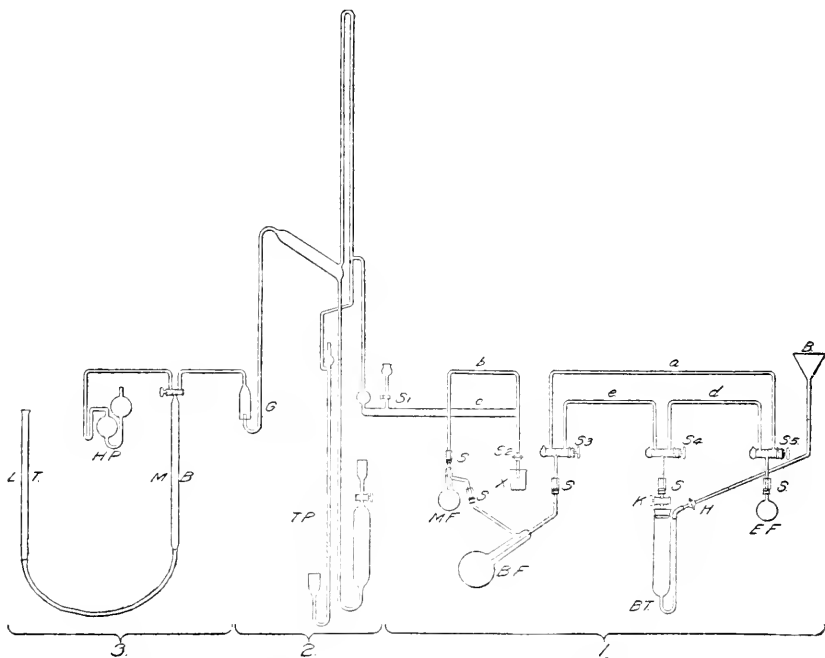


FIG. 1.—Diagram of gas apparatus used in the extraction and analysis of the air confined in butter.

samples of butter fat and butter put up and stored for the investigation which has been described. The apparatus is of glass throughout and consists of three divisions: (1) The system for extracting the gas from the butter tubes, (2) the Töpler pump for transferring the gas so obtained to (3) the usual Hempel apparatus. The rigid and undetachable arrangement of glass tubing and mercury-seal stopcocks comprising the upper part of 1 is conveniently fastened to a wooden frame by means of small, brass pipe bands in the manner seen in Plate CXI, which shows the entire apparatus set up for use. The lower, detachable parts of the extracting system (see fig. 1) consist of the butter tube B. T., the construction and nature of which are described later and which contains the sample

under investigation; the butter flask B. F., of about 1 liter capacity, for retaining the sample after its passage through part of the system; the moisture flask M. F., of 200 c. c. capacity, for retaining the greater part of the moisture liberated from the sample; and a small evacuation flask or globe, E. F., of about the same capacity. These detachable parts are connected with the upper part of the system by means of various mercury seals, S, S.

The operation is begun late in the afternoon of the day before the actual determination by putting the entire division 1 under vacuum and allowing it to stand in this condition overnight. This is done in the following manner:

With the exception of the butter tube B. T., the apparatus is connected as illustrated: The mercury-seal stopcocks  $S_1$ ,  $S_2$ , and  $S_4$  are closed and stopcocks  $S_3$  and  $S_5$  are so turned as to open the system from E. F. through  $a$ , B. F., M. F.,  $b$ , and  $c$  to the Töpler pump T. P. The Töpler pump is now given one stroke, which serves somewhat to exhaust the air confined in the system; a small beaker,  $x$ , filled with concentrated sulphuric acid is brought under stopcock  $S_2$  so that the tube projecting downward from the stopcock is plunged well beneath the surface of the acid, and the beaker is supported in this position. Stopcock  $S_2$  is now cautiously opened until the acid rises to form a long level of drying agent covering the bottom of tube  $c$ , when the influx of acid is stopped. The pump is now worked to its limit of exhaustion (about 0.3 mm. on the McLeod gauge). A turning back and forth of stopcock  $S_5$  accompanied with successive strokes of the pump will evacuate tube  $d$  to stopcock  $S_4$ , and this is followed by turning stopcock  $S_3$  and working the pump to exhaust the tube  $e$ . The entire division 1 is now under exhaust and is allowed to remain so for a considerable length of time, preferably overnight. The next morning, if the gauge indicates that no leakage of air into the system has occurred, the actual determination is made as follows:

The moisture flask M. F. is covered by a beaker which is then packed with cracked ice and salt (sodium chlorid). The butter tube B. T. is connected at  $S_4$  by means of a mercury seal. Funnel B is closed at stopcock H, and filled with a three-fourths saturated sodium-chlorid solution at a temperature of  $50^\circ$  C. A little of this brine solution is allowed to trickle from a pipette into the small side tube of the butter tube B. T. until the latter is completely filled, whereupon it is connected with the funnel tube below H by means of a piece of tight-fitting rubber vacuum tubing. A large glass jar (not shown in the illustration) is now used to cover the butter tube B. T., the base of which rests upon a large rubber stopper with its center removed. The mercury-seal stopcock  $S_5$  is now turned to connect the evacuation flask E. F. with  $d$ , and a turn of  $S_4$  toward  $d$  followed by a closing of the same serves to evacuate the tube from  $S_4$  to the glass stopcock K of the butter tube B. T.  $S_3$  is opened to connect  $e$



with the system B. F., etc. The stopcocks  $S_4$ , K, and H are now all closed. Water at a temperature of  $45^\circ\text{C}$  is poured into the glass jar surrounding B. T. until it immerses the rubber stopper carrying the stopcock K and the rubber connection between the small side tube to B. T. and H. The warmth thus applied to the butter tube at once causes a slight pressure against K and H. H is opened first to allow one or two trapped bubbles of air to escape up toward B and is then closed. K is immediately opened and is soon after followed by the opening of H again. As the material in B. T. melts, a graduated and regulated opening of  $S_4$  permits most of the air confined within the sample to pass over into the system, and the remaining air follows with the melted fat, etc., which passes up, around, and down through  $e$  and trickles into B. F. A too rapid passing of butter containing much curd should be prevented, as it will cause considerable foaming in the butter flask. The warm salt solution flowing in from B displaces the sample from B. T. When the material has been thus removed from B. T. and the level of the salt solution has reached  $S_4$ , this stopcock is closed, followed also by the closing of  $S_3$ . The gas is now transferred from the apparatus by the Töpler pump to the gas-collecting tube G, allowing a few minutes to elapse between strokes of the pump, thus permitting the gas containing moisture not removed by M. F. to become dried by passing over or remaining in contact with the sulphuric acid in  $c$ . The gas is collected over mercury in G and is drawn therefrom into the mercury-filled measuring burette M. B. connected with the leveling tube L. T., from which it is passed over into the Hempel pipettes H. P., where the quantities of carbon dioxide and oxygen in the gas are determined in the usual manner with solutions of potassium hydroxide and alkaline pyrogallol.

#### SPECIAL BUTTER TUBES<sup>1</sup>

These tubes are about 9 inches long and  $1\frac{3}{4}$  inches in diameter, with necks widened somewhat to accommodate a No. 9 rubber stopper carrying a glass stopcock. An ordinary-sized glass tube, bent on itself, leads upward from the base. Each of these tubes when packed will contain about 250 gm. of butter.

These tubes were cleaned, sterilized, and packed with the sample, allowing a very small air space between the surface of the butter and the rubber stopper. Pure, neutral, paraffin oil was poured on the surface of the butter and the stopper was pressed in until the oil in the tube had risen above the stopcock. The stopper was wired down tightly and the stopcock closed. A few cubic centimeters of paraffin oil were then allowed to flow down the side tube. Butter packed in this manner is free from contact with the outside air.

<sup>1</sup>The use of these tubes for packing and storing butter was suggested by Mr. L. A. Rogers, of the Dairy Division.

## EFFECT OF CREAM ACIDITY UPON THE COMPOSITION OF THE AIR IN BUTTER HELD IN STORAGE

The samples, the gas-analysis data of which are given in Tables I, II, and III, were prepared under conditions as nearly identical as possible, the butter having been made at Troy, Pa. In each case the butter was made from 60 pounds of cream taken from one lot, pasteurized at 140° F., and cooled to 48° F. In all three cases the temperature of the buttermilk was 58° F., the quantity of salt added to the butter each time was 12 ounces, and each working was carried to 15 revolutions.

The cream of sample 1 was churned sweet. To the cream of sample 2 was added 15 per cent of the starter, and the churning done at once. Before churning the cream of sample 3, sufficient lactic acid was added to it to make its acidity 0.71 per cent (calculated as lactic acid) by titration.

TABLE I.—Analysis of air extracted from sweet-cream butter

[Calculated to 0° C. and 760 mm. Acidity of cream as lactic acid, 0.11 per cent; salt, 1.21 per cent; curd, 0.58 per cent]

Number of bacteria per gram. <sup>a</sup>	Time stored.			Oxygen.	Carbon dioxide.
	At 0° F.	At 32° F.	At room temperature.		
	<i>Days.</i>	<i>Days.</i>	<i>Hours.</i>	<i>Per cent.</i>	<i>Per cent.</i>
9,050,000.....	0	0	0	<sup>b</sup> 25.15	<sup>b</sup> 2.89
	0	2½	1	22.23	4.51
	0	15	1	15.06	7.58
	0	41	1	9.86	11.91
	0	57	1	5.49	15.24
	81	0	1	25.51	1.49
	81	1	1	22.70	2.02
132,000.....	81	13	1	20.45	2.86
	110	0	5	20.62	2.85
	110	1	1	23.00	2.73
	150	0	2	24.18	1.62
	180	0	1	25.11	0.57

<sup>a</sup> Thanks are due Mr. L. A. Rogers, of the Dairy Division, for the bacteriological work in connection with this investigation.

<sup>b</sup> Analysis of gas extracted from butter as soon as tube was packed.

Samples 1 and 2 were shipped on the afternoon of the same day, arriving in Washington, D. C., shortly before noon of the following day, when the butter was immediately packed into sterilized special glass tubes and small jars and then placed in storage at 0° F. Sample 3 was finished late in the afternoon of the same day on which the preceding samples were made, and did not reach Washington until the second morning after, when the butter was at once packed into tubes and jars and placed in storage under the same conditions as above.

From each of the three samples several tubes were taken and transferred to storage at 32° F. The remainder of the tubes were allowed to continue at a storage temperature of 0° F. From time to time tubes were removed from both temperatures, the gas removed therefrom by

means of the specially devised apparatus, and the quantities of carbon dioxide and oxygen determined.

A perusal of Table I discloses the fact that very little alteration occurred in the composition of the air inclosed in this sample of sweet-cream butter made from cream having an acidity of 0.11 per cent when it was kept for about 6 months at a temperature of 0° F. During this interval practically no diminution of the original oxygen content took place, and the only apparent change to be noted is a probable decrease in the small quantity of carbon dioxide which was known to be present in the butter at the time it was made. An appreciable and progressive change did occur, however, when the butter was kept for nearly two months at a temperature of 32° F. In this case it will be noted that the original oxygen content decreased, while there was a corresponding increase in the initial quantity of carbon dioxide.

Every effort was made to keep the tubes containing this sample, as well as those containing the other differently prepared samples, under comparable conditions. In this connection it may be mentioned that, since it is necessary to surround the tubes with warm water (45° C.) to melt the butter sufficiently to cause it to flow through the apparatus used and that this procedure if carried out immediately upon the removal of the tubes from storage might result in cracking them, the plan was adopted of allowing them to warm up slightly at room temperature for one hour, except in two cases, in which the tubes were intentionally permitted to remain a longer period at room temperature for the purpose of obtaining additional information. It was found that a tube of this butter, when allowed to remain for 5 hours at room temperature after a storage period of 110 days at a temperature of 0° F., contained less oxygen than a corresponding tube of the same sample kept for the same length of time at a temperature of 0° F., and for 1 day at a temperature of 32° F. In measuring the effect of raising the storage temperature to 32° F. on a sample which had been stored at 0° F. for 81 days, it is of interest to note that after holding for 1 day at the higher temperature there is a measurable decrease in the quantity of oxygen known to be present in the sample after the 81 days at the lower temperature, and this effect on the same sample is much more pronounced after holding at the higher temperature for an additional 12 days, or a total of 13 days.

It may be concluded, therefore, that sweet-cream butter prepared as this sample was and containing a considerable number of bacteria will show but little alteration in the composition of the air inclosed in it when it is kept for six months at a temperature of 0° F. A perceptible change, however, occurs when the butter is kept at a temperature of 32° F., and a very noticeable one when it is kept at room temperature. The sample of butter used scored 92 when made and 91 at the end of three months. After a period of six months in storage at a temperature of 0° F. the score was given at 90, there being no trace of any undesirable flavor.

TABLE II.—Analysis of air extracted from butter made from sweet cream churned immediately after the addition of 15 per cent of a commercial starter

[Calculated to °C. and 760 mm. Acidity of cream as lactic acid, 0.25 per cent; salt, 1.19 per cent; curd, 0.59 per cent]

Number of bacteria per gram.	Time stored.			Oxygen.	Carbon dioxide.
	At 0° F.	At 32° F.	At room temperature.		
	Days.	Days.	Hours.	Per cent.	Per cent.
680,000.....	0	7	I	10.89	26.44
	40	0	I	11.64	25.35
	55	0	I	10.84	22.34
	80	0	I	10.70	21.87
160,000.....	100	I	I	10.95	20.92
	150	15	I	8.78	19.27
	205	0	I	9.00	19.50

After the addition of a starter the acidity of the cream from which the butter of sample 2 was made was a little more than twice that of the cream used in the preparation of the sample of sweet-cream butter. A slight but appreciable decrease in the oxygen content of the sample during storage at a temperature of 0° F. was observed, while a perceptible decrease in the carbon dioxide was also manifested. After the sample had been kept for a period at a temperature of 0° F., the effect upon the composition of the air in the butter after standing for several days at a temperature of 32° F. was tabulated, as shown in Table II. This table also shows that a sample of butter made in this manner displays, so far as the composition of the air inclosed in it is concerned, a comparatively slight variation from that observed in the previous case of sweet-cream butter, when both samples are stored at a temperature of 0° F. This sample of butter scored 92 when made, 90 after three months' storage at a temperature of 0° F., and 89 after six and one-half months' storage at the same temperature, there being practically no variation in the flavor during the interval.

The addition of lactic acid to the cream of butter sample 3 before churning brought the total acidity to nearly three times that of the cream used to prepare the foregoing butter of sample 2, and about six and one-half times that of the cream used in making the sweet-cream butter. A pronounced decrease, greater than that observed in either of the two previously given samples of butter, occurred in the oxygen and carbon-dioxide content, even when the butter was stored at a temperature of 0° F., and this decrease was still more marked when it was allowed to remain at a temperature of 32° F. The score of this butter, originally 93, fell to 88 after three months in storage at a temperature of 0° F., and at the end of this interval it had an unclean flavor which was still more pronounced after a period of six months' storage at the same temperature, when the score was 84.

TABLE III.—*Analysis of air extracted from butter made from sweet cream churned immediately after the addition of lactic acid*

[Calculated to 0° C. and 760 mm. Acidity of cream as lactic acid, 0.71 per cent; salt, 0.85 per cent; curd, 0.55 per cent]

Number of bacteria per gram.	Time stored.			Oxygen.	Carbon dioxide.
	At 0° F.	At 32° F.	At room temperature.		
	Days.	Days.	Hours.		
2,050.....	0	6	I	21.58	11.20
0.....	0	32	I	20.53	11.68
0.....	0	48	I	16.70	6.74
0.....	0	62	I	14.93	3.86
0.....	0	80	I	5.95	4.45
0.....	0	82	I	4.17	4.54
0.....	75	0	I	17.30	4.48
0.....	104	0	I	16.94	1.79
0.....	140	0	I	11.74	1.75
0.....	202	0	I	10.84	1.54

Having now determined that the decomposition caused by cream acidity progresses at a temperature of 0° F. in a package of butter and can be measured by an analysis of the gas extracted therefrom, the next step in the investigation of the problem concerning the development of "off flavors" in storage butter involved a series of experiments the purpose of which was to determine whether this measurable decomposition occurs in the fat of the butter itself, in the buttermilk, or in both.

OXIDATION OF PURE BUTTER FAT<sup>1</sup>

The butter fat used in the following determinations was prepared to exclude, so far as possible, by melting, filtering, and washing all ingredients of the butter other than fat and was made from the same lot of cream as the samples of butter B<sub>1</sub> and B<sub>2</sub>, mentioned later. The butter was warmed in a glass vessel to from 32° to 34° C. and allowed to stand, to separate the fat from the greater part of the nonfatty substances. The supernatant fat was then siphoned off, filtered into water at 12° to 14° C., and then thoroughly agitated to granulate it. The fat was then washed several times, salted, and worked on a table worker to the extent of 40 revolutions. The butter fat so prepared was found to contain but 0.05 per cent of protein (total N×6.38). It was packed in absolutely clean and sterile glass jars and also in the special glass tubes for air analy-

<sup>1</sup> The term "pure butter fat" is merely relative. Osborne and Mendel (7) have affirmed that butter fat prepared by centrifugalizing melted butter and pipetting off the clear fat was "entirely free from nitrogen and phosphorus and was devoid of any ash-yielding or water-soluble components." Funk and Macallum (2) have recently challenged this statement as regards nitrogen, since they find that butter fat prepared according to Osborne and Mendel's directions yields easily measured quantities of nitrogen in each of the repeated washings with dilute acid and they conclude that it is very difficult and perhaps impossible completely to free butter fat from nitrogenous substances. McCollum and Davis (4) state that their experiments with butter fat tend to strengthen the conclusion drawn by Funk and Macallum regarding the difficulty of completely freeing the butter fat from nitrogen.

sis. The fat in the jars was covered with a thin layer of paraffin to exclude any action of the atmosphere other than that contained within the material itself. All samples were kept under the same conditions in cold storage at a temperature of 0° F. Samples taken from the lot packed in jars were at once analyzed and, in addition, were scored by Messrs. Corneliuson and Rabild, of the Dairy Division. After intervals of approximately one month, samples were withdrawn from storage, analyzed, and scored. This was continued for several months, during which time a sufficient period had elapsed for the samples to manifest any change which might occur in butter stored for a reasonable length of time.

As may be seen by reference to Table IV, it is certain that no alteration in this sample of butter fat was manifested by the flavor. These samples of nearly pure butter fat showed no physical alteration of any kind after six months or even after one and one-half years in cold storage. There was no development of any characteristic flavor whatsoever, the scoring indicating what might have been expected in case of a material deprived of nearly all its essential ingredients other than fat.

TABLE IV.—Scores of butter fat stored at 0° F.

Age.	Score.	Remarks.	Scorer.
<i>Months.</i>			
1.....	88	Oily, clean flavor...	Corneliuson.
2.....	87	do.....	Do.
3.....	87	do.....	Rabild.
4.....	87	do.....	Do.
5.....	87	do.....	Do.
6.....	87	do.....	Do.
18.....	87	do.....	Do.

As noted earlier in this paper, the following determinations were made to establish a standard as a criterion for judging any change which might occur in the fat of the same lot of butter (whole butter) prepared with varying quantities of nonfatty ingredients (Table V).

TABLE V.—Chemical constants of the butter fat after being nearly freed from the nonfatty ingredients by melting, filtering, and washing and stored at 0° F.<sup>1</sup>

Age.	Reichert-Meißl number.	Iodin number.	Saponification number.	Soluble acids as butyric.	Insoluble acids.	Acetyl value.	Free acid as oleic.
				<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>
Initial.....	30.03	37.30	226.8	5.552	87.54	3.703	0.456
2 months.....	30.17	37.42	226.8	5.572	88.10	3.785	.468
3 months.....	29.84	36.58	226.9	5.483	87.52	3.634	.427
4 months.....	29.67	36.68	226.4	5.140	87.22	3.340	.408

<sup>1</sup> The determinations of chemical constants of the fat incorporated were made by Dr. E. C. Thomssen, formerly of the Dairy Division.

These figures would seem to indicate that very little, if any, chemical change occurred in the fat after having been kept in storage at a temperature of 0° F. for a period of four months, and it was so apparent that no pronounced change could be expected until a longer time had elapsed than is usually practiced in storing butter that this experiment was discontinued. It was apparent from the analysis of the fat that no noteworthy oxidation had occurred therein while the experimental samples were held in storage. An analysis of the air confined within the butter fat is given in Table VI.

TABLE VI.—Analysis of the air in butter fat, stored at 0°F., after being nearly freed from the nonfatty ingredients by melting, filtering, and washing

[Protein, 0.05 per cent; total N X, 6.38; calculated to 0° C. and 760 mm.]

Age.	Total gas.		Total carbon dioxid.		Total oxygen.		Calculated oxygen. <sup>1</sup>
	<i>C. c.</i>		<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>	<i>Per cent.</i>	
<i>Months.</i>	<i>C. c.</i>		<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>
2.....	33.20		0.99	2.98	6.43	19.37	6.44
3.....	27.35		.94	3.44	5.22	19.09	5.28
4.....	29.51		.93	3.15	5.94	20.13	5.72
5.....	38.90		1.38	3.55	7.63	19.61	7.51
12.....	30.81		1.76	5.71	4.27	13.86	5.81
24.....	31.59		.93	2.94	.93	2.94	6.13

<sup>1</sup> After deducting the figure for carbon dioxid from total quantity of gas extracted from the tube, and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

Practically all the carbon dioxid present in the gas extracted from these samples was evidently either in the butter fat at the time of its manufacture or was produced therein within a period of two months after being put into storage. Although the figures would seem to indicate a slight progressive increase in its amount during the storage interval, yet its total amount is small; and in view of the oxygen data obtained it seems to bear little or no relation to the oxygen content. It is very clear, however, that no appreciable oxidation of the nearly pure fat itself occurred during a storage interval of five months; and it was not until after the sample had remained in storage for one year that a slight, measurable oxidation was indicated. In this connection it is thought advisable to note the following general consideration:

Although the iodine numbers obtained for the first and second months and those obtained for the third and fourth months are so close as to resemble duplicate determinations, yet we will take it for granted that the total decrease in the iodine number during the entire period of the investigation is attributable exclusively to the absorption of oxygen by the olein of the fat and not to some one or more of the other factors which, as already indicated earlier in this paper, may influence the data obtained for the iodine number. If we regard 0.72 (the difference between 37.30 and 36.58) as representing the taking up of oxygen by the olein of the fat, the following calculations, based upon this hypothesis, will serve to point out the great improbability of any change in the fat from oxidation during storage at a temperature of 0° F.

Each tube containing the butter fat under investigation in the gas analysis held about 250 gm. of material, corresponding to about 200 gm. of pure fat.<sup>1</sup> The decrease in the quantity of iodine absorbed by a tube would be 1.44 gm., indicating that the fat had absorbed 0.091 gm., or 63.7 c. c. of oxygen. The total quantity of gas incorporated into the sample for the third month, for instance, was only 27.1 c. c., containing approximately but 5.28 c. c. of oxygen in all, and this is obtained from the tube in undiminished quantity in the gas analysis. After one year's storage the material had absorbed only 1.54 c. c. of oxygen, and even after two years' storage the presence of unabsorbed oxygen could still be determined. From the foregoing it will be seen that it is very improbable that any oxidation of pure butter fat occurs during storage at a temperature of 0° F. when the fat is stored for a reasonable length of time. It was decided, however, to make an additional experiment in order to be more certain on this point.

#### BUTTER FAT EXPOSED TO A LARGE SURFACE OF AIR

A sample of butter fat was prepared in the same manner as was the preceding material—by melting, filtering, and washing. In addition, it was given a thorough agitation on the shaking machine with four successive changes of warm water containing 0.5 per cent of hydrochloric acid. The warm butter fat so prepared was allowed to flow through the side tube of the special butter tube filled with pumice fragments until it overflowed through the glass stopcock at the top. The tube was then inverted and the butter fat in the tube permitted to run out. In this manner a small quantity of fat, clinging to the pumice fragments, was exposed to the action of a large quantity of air, a condition just the reverse of that in the previous case. The tubes were then stored at 32° F., a temperature considerably higher than that used in the previous cases. The results are given in Table VII.

TABLE VII.—Oxidation of pure butter fat exposed to the action of a large surface of air at 32° F.

[Calculated to 0° C. and 760 mm.]

Age.	Total gas.		Total carbon dioxide.		Total oxygen.		Calculated oxygen. <sup>2</sup>
	<i>Days.</i>	<i>C. c.</i>	<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>	<i>Per cent.</i>	
30.....		98.60	0	0	19.50	19.78	19.72
61.....		90.30	0	0	16.32	18.07	18.06
100.....		89.40	0	0	15.98	17.87	17.88

<sup>1</sup> Eight tubes of butter fat were put up for this investigation. The average weight of material in each tube was 249.5 gm. The butter-fat content of each tube was approximated as follows: This butter fat was prepared to represent normal butter minus the unfatty constituents (protein, lactose, etc.). The effort was made to incorporate the average quantity of water into it, and salt also was added. With 1 per cent of salt and 16 per cent of water (the maximum) in the butter fat, 250 gm. of the material in the tube would consist of 2.5 gm. of salt, 40 gm. of water, and 207.5 of fat ("about 200 gm."). Taking any smaller percentage of water than 16 would increase the percentage of fat, which would, of course, call for a greater absorption of iodine than 1.44 gm., expressing the taking up of a greater quantity of oxygen than 63.7 c. c. This would have the effect of making still more pronounced the point here brought out.

<sup>2</sup> After deducting the figure for carbon dioxide from the total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.



From the above-mentioned data it will be seen that but a slight oxidation of the fat occurred during a storage interval of more than three months at a temperature of 32° F., even when the sample was kept under conditions decidedly more favorable, in comparison with the preceding one, to permit any pronounced oxidation. It would appear, therefore, that any oxidation of pure butter fat kept in storage at a temperature of 0° F. for a reasonable length of time, if it occurs at all, must be extremely slight. The results of experiments already conducted, however, have shown that a progressive oxidation in whole butter may occur while held in storage at a temperature of 0° F.

The question, therefore, arises whether there occurs an oxidation progressing in some one or more of the nonfatty constituents of whole butter. In the attempt to clear up this point the following experiments were conducted.

#### OXIDATION OF NONFATS

The butter samples used in the two following experiments were made in the experimental creamery at Troy, Pa., from the same lot of cream as was the preceding sample of butter fat stored at a temperature of 0° F. The cream was pasteurized in a continuous pasteurizer at a temperature of 165° F., and was ripened with a pure culture. The acidity of the cream at the time of churning was 0.40 per cent (calculated as lactic acid). The butter in the churn was washed until the wash water was just clear. One half of the butter in the churn was removed and was designated as "normally washed butter." The other half, which remained in the churn, was now given an additional copious washing in four changes of water and designated as "excessively washed butter."

The sample designated in the experiments as "unwashed butter" was prepared from a different lot of cream, which was pasteurized and ripened under the same conditions as indicated above. It was ripened to an acidity of 0.51 per cent (calculated as lactic acid), cooled to 7½° C. (45.5° F.), held overnight, during which the acidity rose to 0.65 per cent, and then churned. The buttermilk was drawn off and the butter allowed to remain unwashed, so as to contain the greatest amount of nonfatty ingredients of all three samples.

Since it was desired to have the three foregoing samples differ from one another only with respect to their buttermilk content, care was taken to prepare them otherwise in identically the same manner. Each was worked on a table worker to the extent of 40 revolutions, to incorporate a large quantity of air. They were then packed in clean and sterile glass jars, and also in the special glass tubes for air analysis. The butter in the jars was covered with a thin layer of paraffin to exclude any action of the atmosphere other than that confined within the material itself.

The appearance of undesirable flavors in stored butter has often been attributed to the use of either impure salt or water, or both, so this contingency was avoided by the use, in all cases, of chemically pure sodium chlorid and distilled water.

The samples were shipped by express to Washington, D. C., where they were kept in cold storage at a temperature of 0° F. Samples taken from the various lots packed in the jars were at once analyzed, and, in addition, were scored by Messrs. Corneliuson and Rabild, of the Dairy Division. After intervals of approximately one month, samples were withdrawn from storage, analyzed, and scored. This was continued for several months, during which time a sufficient period had elapsed for the samples to manifest any change which might occur in butter stored for a reasonable length of time. Of the three samples, designated for convenience as "excessively washed butter," "normally washed butter," and "unwashed butter," the first two will be given and discussed in conjunction (Tables VIII and IX).

TABLE VIII.—Scores of excessively washed butter, with low content of nonfatty ingredients, stored at 0° F.

[Protein, 0.50 per cent. Total N×6.38]

Age.	Score.	Remarks.	Scorer.
<i>Months.</i>			
1.....	90	Good, but trifle stale.....	Corneliuson.
3.....	87	.....do.....	Do.
5.....	88	.....do.....	Do.
6.....	87	.....do.....	Do.

TABLE IX.—Scores of normally washed butter, with normal content of nonfatty ingredients, stored at 0° F.

[Protein, 0.57 per cent. Total N×6.38]

Age.	Score.	Remarks.	Scorer.
<i>Months.</i>			
1.....	91	Flavor good.....	Corneliuson.
2.....	89	Trifle stale.....	Do.
3.....	87	Aroma good, trifle stale.....	Rabild.
5.....	88	Flavor good.....	Corneliuson.
6.....	88	.....do.....	Do.

The keeping qualities of the two foregoing samples were practically the same, as shown by the scoring. The determination of the chemical constants gave the data in Tables X and XI.

TABLE X.—Chemical constants of the fat of excessively washed butter, with low content of nonfatty ingredients, stored at 0° F.

[Protein, 0.50 per cent. Total. N×6.38]

Age.	Reichert-Meissl number.	Iodin number.	Saponification number.	Soluble acids as butyric.	Insoluble acids.	Acetyl value.	Free acid as oleic.
Initial.....				<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>
2 months.....	30.03	37.30	226.8	5.552	87.54	3.703	0.456
4 months.....	29.83	36.52	226.4	5.623	87.63	3.578	.458
	29.89	36.42	225.9	5.130	87.58	3.535	.413

TABLE XI.—Chemical constants of the fat of normally washed butter, with medium content of nonfatty ingredients, stored at 0° F.

[Protein, 0.57 per cent. Total, N×6.38]

Age.	Reichert-Meissl number.	Iodin number.	Saponification number.	Soluble acids as butyric.	Insoluble acids.	Acetyl value.	Free acid as oleic.
Initial.....	30.03	37.30	226.8	<i>Per cent.</i> 5.552	<i>Per cent.</i> 87.54	3.703	0.456
2 months.....	30.64	36.39	226.5	6.072	87.93	3.581	.425
3 months.....	30.16	36.08	226.1	5.400	87.56	3.942	.459
5 months.....	29.78	36.52	226.6	5.150	87.44	3.323	.412

There is practically no variation in these figures from those obtained in the foregoing determination of chemical constants with the nearly pure butter fat standard. Evidently the fat in these two samples of butter underwent little or no chemical change, owing to the presence of either the confined air or the other nonfatty components. The analysis of this confined air, however, gave figures which differed considerably from those obtained in the analysis of the air confined within the samples of the butter fat itself (Table XII).

TABLE XII.—Analysis of air in excessively washed butter, with low content of nonfatty ingredients, stored at 0° F.

[Calculated to 0° C. and 760 mm. Protein, 0.50 per cent. Total, N×6.38]

Age.	Total gas.		Total carbon dioxide.		Total oxygen.		Calculated oxygen. <sup>1</sup>
	<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>	<i>Per cent.</i>	
<i>Months.</i>							<i>C. c.</i>
2.....	31.88	4.48	14.05	5.52	17.31	5.50	
3.....	42.50	2.77	6.52	6.47	15.23	7.95	
4.....	26.57	2.01	7.57	3.39	12.76	4.91	
5.....	26.28	1.94	7.38	3.24	12.33	4.87	
14.....	30.55	1.74	5.70	1.84	6.03	5.78	

<sup>1</sup> After deducting figure for carbon dioxide from total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

TABLE XIII.—Analysis of air in normally washed butter, with medium content of nonfatty ingredients, stored at 0° F.

[Calculated to 0° C. and 760 mm. Protein, 0.57 per cent. Total, N×6.38]

Age.	Total gas.		Total carbon dioxide.		Total oxygen.		Calculated oxygen. <sup>1</sup>
	<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>	<i>Per cent.</i>	
<i>Months.</i>							<i>C. c.</i>
2.....	27.88	1.85	6.64	5.26	18.87	5.21	
3.....	29.09	3.97	13.24	4.34	14.47	5.20	
4.....	26.26	3.45	13.14	3.72	14.17	4.56	
14.....	28.94	3.90	13.47	2.41	8.33	5.01	

<sup>1</sup> After deducting figure for carbon dioxide from total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

There is no great difference in the total quantity of carbon dioxide to be observed between these samples of excessively washed butter and what is considered to represent normally washed butter. It is to be noted that there is very little difference in the protein content of these two

samples. The high point (14.05 per cent) for carbon dioxide reached in the first case is about the same as that reached in the second (13.47 per cent), while there is no wide variation in the oxygen content of the two samples. The great decrease in the percentage of carbon dioxide in the former case occurs in the interval between the second and third months, after which this decreased percentage remains fairly constant. This decrease in the original percentage of carbon dioxide is also accompanied with a pronounced decrease in the percentage of oxygen. In the latter case the percentage of carbon dioxide increases to its maximum after the sample has been three months in storage, after which it remains fairly constant. It is to be noted, however, that the total amount of oxygen originally present in these samples of butter containing a certain proportion of buttermilk undergoes a markedly progressive decrease during the interval that the butter is kept in storage at a temperature of 0° F.

A survey of the data obtained from the sample of unwashed butter is of additional interest in this connection (Table XIV).

TABLE XIV.—Scores of unwashed butter, with high content of nonfatty ingredients stored at 0° F.

[Protein 0.90 per cent. Total N×6.38]

Age.	Score.	Remarks.	Scorer.
<i>Moniks.</i>			
1.....	92	Oily, mottled.....	Corneliuson.
2.....	89	Oily, unclean.....	Rabild.
3.....	87	.....do.....	Corneliuson.
5.....	85	Slightly fishy.....	Do..
6.....	86	Stale, fishy, sour.....	Do.

The progressive development of "off flavor" in this sample of butter, so prepared as to contain a greater quantity of buttermilk than either of the two foregoing samples, was remarkable. Since this butter had been prepared from a different lot of cream, it was necessary to establish a new fat standard of constants. The butter fat for this purpose was prepared from the same lot of cream as was the butter, and it was packed and stored in the same manner as that given for the previously mentioned sample of butter fat. The results are given in Tables XV and XVI.

TABLE XV.—Chemical constants of butter fat stored at 0° F after being nearly freed from the nonfatty ingredients by melting, filtering, and washing

Age.	Reichert-Meissl number.	Iodin number.	Saponification number.	Soluble acids as butyric.	Insoluble acids.	Acetyl value.	Free acid as oleic.
Initial.....	26. 16	41. 91	226. 3	<i>Per cent.</i> 5. 275	<i>Per cent.</i> 86. 93	3. 365	<i>Per cent.</i> 0. 210
2 months.....	26. 90	41. 40	225. 9	5. 147	87. 49	3. 579	. 220
3 months.....	26. 71	40. 76	226. 2	5. 263	87. 65	3. 260	. 222
5 months.....	26. 93	40. 79	226. 5	5. 220	87. 55	3. 335	. 214
6 months.....	26. 84	40. 88	225. 1	5. 166	87. 38	3. 397	. 225

TABLE XVI.—*Chemical constants of the fat of unwashed butter, with high content of non-fatty ingredients, stored at 0° F.*

[Protein 0.90 per cent. Total N×6.38]

Age.	Reichert-Meißl number.	Iodin number.	Saponification number.	Soluble acids as butyric.	Insoluble acids.	Acetyl value.	Free acid as oleic.
<i>Months.</i>				<i>Per cent.</i>	<i>Per cent.</i>		<i>Per cent.</i>
1.....	26.28	41.80	226.6	5.29	87.1	3.90	0.205
2.....	26.76	40.40	226.4	5.06	86.66	3.795	.210
3.....	26.90	40.81	226.3	5.32	87.02	3.381	.226
5.....	26.83	40.13	226.5	5.27	87.44	3.331	.231
6.....	26.84	40.30	225.0	5.17	86.98	3.294	.226

The same comments are here to be made as in the case of the previous sample of nearly pure butter fat. No noteworthy chemical change had occurred in this sample of butter fat after having been kept in storage at a temperature of 0° F. for a period of six months. With respect to the fat taken from the sample of butter so prepared as to contain the greatest number of constituents in addition to the fat, the same observations are here to be made as in the previous cases of two different lots of butter containing smaller numbers of nonfatty constituents. The chemical constants here show little or no variation from those obtained with the nearly pure butter fat, and there is apparently no chemical change in the fat of butter prepared with a still greater number of substances in addition to the fat, owing either to the presence of these substances or to the presence of the confined air. An analysis of this confined air, however, gives some very striking data (Table XVII) when compared with those obtained in the foregoing samples.

TABLE XVII.—*Analysis of air in unwashed butter, with high content of nonfatty ingredients, stored at 0° F.*

[Calculated to 0° C. and 760 mm. Protein 0.90 per cent. Total N×6.38]

Age.	Total gas.		Total carbon dioxide.		Total oxygen.		Calculated oxygen. <sup>1</sup>
	<i>C. c.</i>	<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>	<i>Per cent.</i>	<i>C. c.</i>	
2.....	29.26	9.19	31.41	0.71	2.43	4.01	
3.....	28.23	8.94	31.67	.62	2.20	3.86	
4.....	29.15	8.13	27.89	.47	1.61	4.20	
5.....	38.19	9.97	26.12	.37	.97	5.64	
15.....	33.28	7.41	22.26	.37	1.11	5.17	

<sup>1</sup> After deducting the figure for carbon dioxide from the total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

The maximum content of carbon dioxide (31.67 per cent) in this sample of unwashed butter was noticed after a storage period of three months, at about which time the characteristic “off flavor” became distinctly noticeable. At the end of two months there was very little oxygen in the sample; yet even this shows a perceptible decrease during the storage interval.

It has been indicated in the foregoing experiments that the quantity of carbon dioxide occurring in the gas inclosed in a package of stored butter is proportional to the amount of nonfatty ingredients incorporated into the material. There is also a more or less pronounced decrease in the oxygen content during the storage period. The following additional experiment was made with the view of confirming this relative change in the percentages of carbon dioxide and oxygen, and especially to determine the quantities of these gases occurring in unwashed butter at the time of its manufacture, since all the above-described analyses were made upon the samples after an interval of two months in storage.

For this purpose some unwashed butter was prepared from cream pasteurized at 145° F. for 20 minutes, and, as in the other cases, ripened with a pure culture. The cream was ripened to an acidity of 0.45 per cent (calculated as lactic acid), cooled to 7° C. (44.6° F.), held overnight, during which the acidity rose to 0.67 per cent, and then churned. The buttermilk was drawn off and the butter allowed to remain unwashed. The butter was then salted with chemically pure salt and worked on a table worker. This butter contained 4.72 per cent of sodium chlorid and 0.56 per cent of protein (total N×6.38). The butter was then packed into the special glass tubes for air analysis.

The gas in the first sample was extracted therefrom and analyzed as soon as possible after the butter was made—that is, 1½ hours. The remaining samples were kept at room temperature, but in the dark (Table XVIII).

TABLE XVIII.—Analysis of air from a second sample of unwashed butter kept at room temperature but in the dark

[Calculated to 0° C. and 760 mm. Protein 0.56 per cent. Total N×6.38]

Age.	Total gas.		Total carbon dioxide.		Total oxygen.		Calculated oxygen. <sup>1</sup>
	C. c.		C. c.	Per cent.	C. c.	Per cent.	
1½ hours . . . . .	37.7		7.5	19.89	7.7	20.42	6.0
2 days . . . . .	33.4		7.4	22.16	5.6	16.77	5.2
7 days . . . . .	33.8		7.5	22.19	5.1	15.09	5.3
14 days . . . . .	35.8		8.2	22.91	3.8	10.61	5.5

<sup>1</sup> After deducting the figure for carbon dioxide from the total quantity of gas extracted from the tube and assuming that the residual gas is pure air—that is, approximately one-fifth oxygen.

Most of the carbon dioxide appears to have existed in the butter as soon as the manufacture of the material was completed. It also appears to increase somewhat in quantity during a period of two weeks. The oxygen figures show in a striking manner the decrease in the initial quantity of this gas present in the butter, and it is apparent that it has decreased to practically one-half this quantity after being kept two weeks at room temperature in the dark.

The question now arises whether there exists in the samples of butter fat the same homogeneous distribution of air bubbles as in the case of those samples of butter containing the varying quantities of nonfatty ingredients, for it is conceivable that the air incorporated into the butter fat may occur mostly in large pockets, while the other samples may contain, in addition, a certain amount of the total air inclosed within the particles of curd, lactose, etc.

In the first case it is reasonable to suppose that a smaller surface of material would be exposed to the influence of the air than in the second; yet it is improbable that this would alter the basic facts, since the analytical data obtained in the experiments indicate that the particles of nonfatty ingredients inclosing the air are more readily attacked by the oxygen therein than the fat itself. However, to obtain further confirmatory data on this point—that is, that the nonfatty constituents of butter are more readily attacked by the oxygen of the air incorporated into the material than the fat itself—the following experiments were conducted.

BUTTERMILK EXPOSED TO A LARGE SURFACE OF AIR

Several of the special butter tubes were filled with large fragments of cracked and ignited pumice. The pumice of one lot of tubes was impregnated with the buttermilk from butter made from pasteurized cream acidified to 1 per cent with lactic acid before churning. The pumice of each tube of a second lot was treated with 10 c. c. of a 1 per cent solution of lactic acid. The tubes of these two lots were kept at a temperature of 32° F. At various times tubes from each were removed from storage and an analysis of the air in them was made. The analytical data obtained are given in Table XIX.

TABLE XIX.—*Oxidation of acid-cream buttermilk and of lactic acid exposed to the action of a large surface of air at a temperature of 32° F.*

Acid buttermilk.			Lactic acid.		
Period at 32° F.	Oxygen.	Carbon dioxide.	Period at 32° F.	Oxygen.	Carbon dioxide.
Days.	Per cent.	Per cent.	Days.	Per cent.	Per cent.
4½.....	17.67	2.37	30	20.70	0
26.....	0	34.37	60	21.07	0
62.....	0	31.76	98	21.01	0

The change in the composition of the air in contact with the acid buttermilk was very marked during a storage interval of only 26 days when this sample was kept at a temperature of 32° F. From a total percentage of 17.67 found to be present in the acid buttermilk when the material was 4½ days old, the oxygen content fell to zero during the period between this time and 26 days. The carbon-dioxid content of the buttermilk, initially small in quantity, rapidly increases to a maxi-

mum, from which point it begins to decrease. It is also very clear from the control experiment given in Table XIX that the change in the composition of the air inclosed in the material is not caused by decomposition of the lactic acid itself or to any action of this acid upon the particles of pumice.

It has already been shown in this paper that the acidity of the cream from which the butter is made has a direct influence on the change, during storage at a temperature of  $0^{\circ}$  F., in the composition of the air incorporated into the butter at the time of its manufacture. It has also been shown that but a slight change is to be observed in the composition of the air from a tube containing a small quantity of pure butter fat exposed to the action of a large and confined surface of air while the fat was kept at a temperature of  $32^{\circ}$  F. It has likewise been demonstrated that practically no change in the composition of the air occurs when pure butter fat is exposed to the action of about the same amount of air as is usually present in normal butter while it is stored at a temperature of  $0^{\circ}$  F. As it has been proved by an analysis of the air from a sample of sweet-cream butter made from cream of low acidity that this kind of butter suffers very little, if any, measurable decomposition during a period of six months in storage at a temperature of  $0^{\circ}$  F., and having also proved by other experiments that a decomposition of the fat of whole butter stored at a temperature of  $0^{\circ}$  F. for the same length of time is practically excluded, it is a logical conclusion that the particles of buttermilk inclosed in a sample of sweet-cream butter made from cream of low acidity likewise suffers little, if any, measurable decomposition when the butter is stored at a temperature of  $0^{\circ}$  F. It was decided, however, to settle this point definitely by experiment.

For this purpose the pumice of a third lot of tubes was impregnated with buttermilk from sweet-cream butter made from pasteurized cream having an acidity of 0.108 per cent (calculated as lactic acid). The acidity of the cream in this case was practically the same as that of the cream from which the foregoing sample of sweet-cream butter was made. This last lot of tubes containing the sweet-cream buttermilk was kept at a temperature of  $0^{\circ}$  F. (Table XX).

TABLE XX.—Oxidation of sweet-cream buttermilk exposed to the action of a large surface of air at  $0^{\circ}$  F.

Period at $0^{\circ}$ F.	Oxygen.	Carbon dioxid.
Days.	Per cent.	Per cent.
35.....	20.92	0
65.....	20.93	0
270.....	20.25	0

That the sweet-cream buttermilk underwent practically no change in storage at a temperature of  $0^{\circ}$  F. is shown by the foregoing data.



## SUMMARY AND CONCLUSIONS

The composition of the air confined within a package of pasteurized sweet-cream butter known to contain bacteria and made from cream having an acidity of 0.11 per cent (calculated as lactic acid) showed little or no variation from its original composition after successive periods in storage, aggregating six months, at a temperature of 0° F. A small quantity of the buttermilk from butter made from pasteurized sweet cream having the same low degree of acidity as the cream above mentioned, when exposed to the influence of a very large and confined surface of air, appeared to have little, if any, effect upon the original composition of the air when the buttermilk was stored for nine months under like conditions of temperature. A portion of this same sample of sweet-cream butter when kept at a temperature of 32° F. showed a decided change in the original composition of the inclosed air, a change which was still further increased when the butter remained for a short time at room temperature. This change in the composition of the air originally incorporated into the butter was expressed by a decrease in the percentage of oxygen and a corresponding increase in the percentage of carbon dioxide. This sample of sweet-cream butter still possessed a good score after six months' storage at a temperature of 0° F., there being no indication of any undesirable flavor.

The change in the composition of the air initially inclosed within a package of butter made from sweet cream and churned immediately after the addition of 15 per cent of a commercial starter showed but little variation from that observed in the sample of sweet-cream butter when the two samples were kept under comparable conditions, both being in storage at a temperature of 0° F., although the acidity of the cream in the first case was somewhat higher (0.25 per cent) than that of the cream from which the sweet-cream butter was made. This sample of butter also displayed good keeping qualities during its storage period of nearly seven months at a temperature of 0° F.

The composition of the air inclosed within a package of butter made from sweet cream and churned immediately after the addition of lactic acid, the total acidity of the cream being about six and one-half times greater than that of the cream from which the sweet-cream butter was made, showed pronounced variations from its original composition during successive periods of storage at a temperature of 0° F. These variations were still greater when the sample was allowed to stand at a temperature of 32° F. In this case there was a considerable and a progressive decrease in the original oxygen content, as well as in the original carbon-dioxide content. A small quantity of the buttermilk from butter made from pasteurized sweet cream and churned immediately after the addition of lactic acid, when exposed to the action of a very large and confined surface of air under the same temperature conditions, showed precisely the

same phenomena with respect to alteration in the original air composition. The oxygen content of the confined air had entirely disappeared within a month's time. The carbon-dioxid content, originally 2.37 per cent, had increased to more than 34 per cent within the same interval, after which time it had begun to decrease. The flavor of this butter, which was prepared from pasteurized sweet cream and churned immediately after the addition of lactic acid, was somewhat unclean after a storage period of only three months at a temperature of 0° F., and decidedly so after being in storage for six months under the same conditions.

Further, it has been indicated by the investigation pursued with pasteurized, ripened-cream butter through the successive steps from nearly pure butter fat to samples of butter containing varying quantities of ingredients other than fat and, finally, to samples containing the greatest quantity of protein, lactose, etc., and stored for a reasonable length of time (six months) at a temperature of 0° F., that the amount of carbon dioxid inclosed in a package of the material is directly proportional to the quantity of these ingredients contained therein. It has been shown that this quantity of carbon dioxid may increase during the earlier part of the storage period, followed by a decrease during the latter part. It is also of especial significance, perhaps, that the oxygen content of the gas in the material undergoes a marked and striking decrease during the interval that the samples of butter containing the varying amounts of constituents other than fat are retained in storage, and that this decrease is likewise proportional to the amount of acid and ingredients other than fat contained in the butter.

The fat of butter made from pasteurized cream, on the contrary, undergoes no apparent oxidation during the same storage period when kept at a temperature of 0° F. It is only when a substance like pumice, which may have catalytic properties, is impregnated with a small amount of butter fat and exposed to the action of a large amount of air while kept at a temperature of 32° F. that a very slight oxidation is noticeable.

The results of the investigations may be summed up as follows:

(1) The development of undesirable flavors in butter held in cold storage at a temperature of 0° F. is not dependent upon an oxidation of the fat itself.

(2) The production of "off flavors" so commonly met with in cold-storage butter is attributable to a chemical change expressed through a slow oxidation progressing in some one or more of the nonfatty substances occurring in the buttermilk.

(3) The extent of this chemical change is directly proportional to the quantity of acid present in the cream from which the butter was prepared.

(4) The quantity of carbon dioxid present in cold-storage butter appears to have a certain relation to the quantity of buttermilk in the butter. During storage this quantity of carbon dioxid may increase to a maximum followed by a progressive decrease.

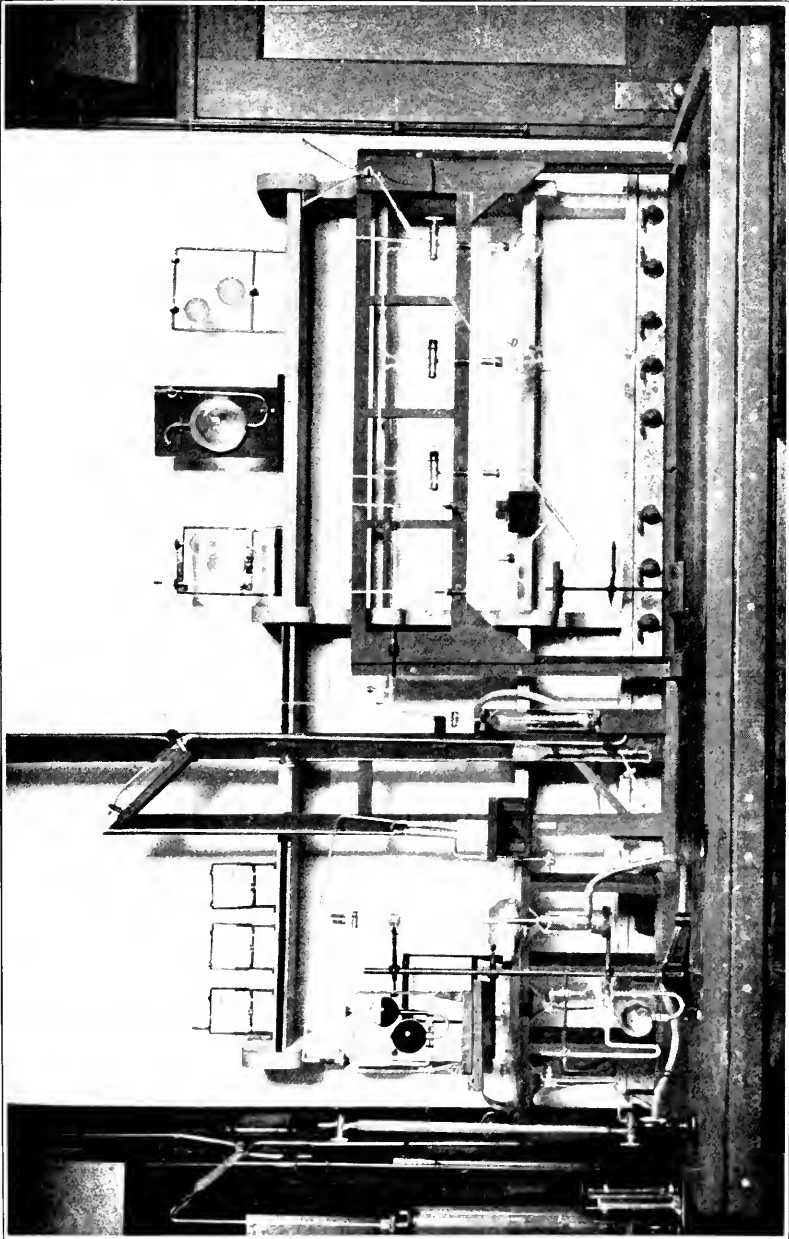
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PLATE CXI

Gas apparatus used in the extraction and analysis of the air confined in butter.

(952)





# BACTERIOLOGICAL STUDIES OF A SOIL SUBJECTED TO DIFFERENT SYSTEMS OF CROPPING FOR TWENTY-FIVE YEARS

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## INTRODUCTION

During the past few years a number of soil biologists have reported their findings regarding the effects of different agricultural practices upon the bacteria of the soil. The majority of these investigations have been concerned with determining the gross effects of a particular treatment upon the physiological activities of the flora as a whole. Such factors as continuous cropping, rotational systems, cultural methods, application of chemical fertilizers, and manures have been studied. Among the more recent workers reporting such investigations in the United States may be mentioned King and Doryland (10),<sup>1</sup> Stevens and Withers (18), Stewart and Greaves (19), Lyon and Bizzell (12), Temple (20), Jensen (9), Given and Willis (6, 7), Brown (2, 3, 4), Hill (8), Allen and Bonazzi (1), Wright (22), and McBeth and Smith (13).

It is not necessary to give any extensive review of the work that has been done as the papers referred to above contain full summaries of the results thus far obtained. Special attention may be called to the review given by Temple (20) as to the effect of stable manure, by Hill (8) as to the effect of other organic materials, and by Lyon and Bizzell (12) as to the effect of different growing crops. The available data leave little doubt that certain of the above-mentioned factors do exert a marked effect upon soil organisms. In very few instances, however, has any serious effort been made to ascertain just how such factors exert their influence. In most instances the treatment in question has been in operation a comparatively short time. Brown (3), for example, studied the effects of a 4-year rotation while the fourth crop was still on the soil, or before the cycle was completed the first time. It is true that the quantities of nitrate nitrogen have been determined *in situ*, following long-continued cropping systems. With our present very limited knowledge as to the demands any particular crop makes upon soil nitrates, such information gives us little insight into nitrate formation. Allen and Bonazzi (1) carried out a few laboratory experiments, following a long-continued treatment, but obtained such irregular results that they regarded them of little value. Given and Willis (6, 7) have also re-

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<sup>1</sup> Reference is made by number to "Literature cited," p. 974-975.

ported a limited number of experiments following 30 years of continuous treatment.

It was with the ultimate object of attempting a study of the fundamental causes for variations in certain changes in soil nitrogen that this work was undertaken. Before it could be begun, however, it was first necessary to determine the existence of differences under our conditions. Furthermore, it seemed desirable to compare the relative effect of long-continued treatment with that of the shorter periods reported by others.

The Missouri Experiment Station possesses a series of fertility plots that offered exceptional opportunity for making the above-mentioned studies. The plots cover a rather wide range and have just passed their twenty-fifth year of continuous treatment as outlined in the original project. Some very valuable data as to the effect upon fertility as measured by crop-producing power have been obtained. It would seem as if 25 years would have so materially changed the micro-organic life therein that such could readily be detected, provided such differences were actually brought about. With such material upon which to work it would seem that the verity of marked differences in similarly treated soil reported by others could be established.

The data herein reported have been obtained from some of the plots that have given the most marked differences in yield, as it was believed such plots would offer the best material upon which to work. The work, however, is concerned only with demonstrating the existence of differences and offers only one or two suggestions as to the actual cause of such differences. We hope to be able to throw more light upon this particular field at a later date. Furthermore, the particular data here reported have to do only with bacterial numbers and with ammonia and nitrate-forming abilities.

Certain facts, which, we believe, we have very clearly demonstrated, have been of value to us in directing further work. It is with the hope that the data may be of similar value to others that we present them in this paper.

#### PLOTS STUDIED

The fertility plots of the Missouri Experiment Station are located on the soil type classed as Putnam silt loam. They were first planted to the present system in 1889 and, with few irregularities, have received the same treatment as outlined. Each plot consists of one-tenth acre and is surrounded by an alley 3 feet wide. In selecting from the large number of plots the few that could be handled in our work, an effort was made to include as wide a variation of treatment as possible and at the same time to avoid inherent soil differences in order that the work might be comparative.

The plots studied are, with the exception of No. 29 and 30, located just at the crest and on the eastern slope of a gentle rise. Plots 29 and



30 are of a slightly different texture, being located on the western slope of the same rise. For this reason they are omitted from the general scheme and are compared with each other only. The greatest distance between any two plots (No. 1 and 23) is only about 85 yards. It was impossible to get the specially treated plots that we wished to study located any closer together. It is not believed, however, that any difference due to character of soil, location, etc., could materially affect the major differences reported. The plots studied, treatment received, and yields are given in Table I. It will be noted that the plots studied during 1914 varied somewhat from those of the previous year. This was necessary because of a change in treatment in certain plots beginning in 1914. In the tabulated data, plot 1 always includes the data obtained from plot 20 (a duplicate). Similarly plot 10 includes data secured from plot 21 (a duplicate).

Where stable manure has been applied, it has been an annual application averaging 6.7 tons per acre on all plots except No. 1, which received 7 tons. The chemicals were applied annually on plots 2 and 3 in the form of sodium nitrate, potassium chlorid, and acid phosphate in quantities sufficient to supply nitrogen, potassium, and phosphorus for a full yield of the particular crop. In the case of wheat this was for a yield of 40 bushels. The rotation consisted of corn, oats, wheat, clover, timothy, and timothy. The other plots have been annually planted to the specific crop mentioned.

TABLE I.—Cropping system, treatment, and yields of the various fertility plots studied at Columbia, Mo.

Plot No.	Cropping system.	Treatment.	Year studied.	Yield.				
				1914	1913	1909-1913	1904-1913	1889-1913
1	6-year rotation <sup>a</sup> .....	Stable manure.....	1914	1,326 pounds.....	3,887 pounds.....	12.83 bushels.....	16.38 bushels.....	17.99 bushels.
2	Continuous wheat.....	Chemicals.....	1914	29.32 bushels.....	17.01 bushels.....	.....	.....	.....
3	6-year rotation.....	.....do.....	1914	964 pounds.....	2,964 pounds.....	.....	.....	.....
9	Continuous wheat.....	None.....	1914	20.09 bushels.....	8.83 bushels.....	6.41 bushels.....	6.54 bushels.....	9.56 bushels.
10	.....do.....	Stable manure.....	1914	26.37 bushels.....	16.90 bushels.....	12.21 bushels.....	12.47 bushels.....	16.72 bushels.
13	6-year rotation.....	None.....	1913-14	377 pounds.....	1,568 pounds.....	.....	.....	.....
17	Continuous corn.....	.....do.....	1913-14	38.78 bushels.....	6.96 bushels.....	10.68 bushels.....	10.68 bushels.....	20.35 bushels.
18	.....do.....	Stable manure.....	1913-14	34.89 bushels.....	19.22 bushels.....	19.64 bushels.....	24.54 bushels.....	35.78 bushels.
20	6-year rotation.....	.....do.....	1913	1,177 pounds.....	4,810 pounds.....	.....	.....	.....
21	Continuous wheat.....	.....do.....	1913	38.41 bushels.....	17.44 bushels.....	14.35 bushels.....	15.82 bushels.....	18.66 bushels.
22	Continuous timothy.....	.....do.....	1913-14	1,482 pounds.....	3,042 pounds.....	4,388 pounds.....	4,345 pounds.....	5,006 pounds.
23	.....do.....	None.....	1913-14	910 pounds.....	1,339 pounds.....	2,075 pounds.....	2,264 pounds.....	2,853 pounds.
29	Continuous wheat.....	Stable manure <sup>b</sup> .....	1913	29.56 bushels.....	16.79 bushels.....	11.87 bushels.....	12.59 bushels.....	15.61 bushels.
30	.....do.....	.....do.....	1913	28.17 bushels.....	22.53 bushels.....	12.66 bushels.....	14.96 bushels.....	19.58 bushels.

<sup>a</sup> The 6-year rotation plots were in clover during 1913 and timothy during 1914.<sup>b</sup> Stable manure since 1908.

## EXPERIMENTAL METHODS EMPLOYED

Practically all the methods here mentioned have been severely criticised during the past few years, particularly by Löhnis and Green (11), Allen and Bonazzi (1), and Noyes (14). However, in our laboratory the methods described below have proved, for the object in view, equal or superior to any suggested prior to the beginning of this work.

Samples for the various analyses were taken with a 1½-inch soil auger. Ten to fifteen samples were collected from each plot to the depth of the soil, which was about 10 inches. The cores of soil were taken uniformly all over the plot, avoiding close proximity to the surrounding alleys. They were placed immediately in sterile Mason jars in order to prevent loss of moisture and to avoid contamination, so far as possible. The samples were then brought to the laboratory as soon as possible, where, under aseptic conditions, the soil was passed through a 2-mm. sieve and thoroughly mixed. Samples were immediately taken for quantitative analyses and for moisture determinations.

For moisture determinations 50 gm. of soil were dried at 110° C. for two hours. To determine the water-holding capacity, 50 gm. were placed in a carbon filter containing a perforated porcelain bottom and a measured quantity of water poured on top. The water was permitted to percolate through the soil. The process was repeated two or three times. From the amount of water absorbed plus the quantity lost in drying, the water-holding capacity, expressed in grams of water held per 100 gm. of dry soil, was determined. Data obtained by these methods are, of course, not absolute. But the process possesses two essentials: Quickness of manipulation and comparativeness. Since slight differences in water content, when near the optimum, exercise but little influence upon bacterial activity, it is believed the error introduced is not appreciable.

Quantitative analyses were made by carefully weighing 1 or 2 gm. of soil, placing it in 98 or 99 c. c. of sterile water and shaking vigorously for one minute. From this suspension dilutions were made in the ordinary way. Finally, 1 c. c. was placed in each of three sterile Petri dishes and thoroughly mixed with 10 c. c. of Temple's agar (20). The dishes were then incubated for one week at room temperature and all colonies counted with the aid of a hand lens. If one of the dishes varied widely from the other two it was discarded. The same was true if dishes were overrun by spreaders or molds. The results are reported in millions of bacteria per gram of dry soil.

The ammonia- and nitrate-forming experiments were carried out by thoroughly mixing into fresh soil (the equivalent of 100 gm. of dry soil) sterile cottonseed meal containing 60 mgm. of nitrogen. This was placed in a sterile 500 c. c. wide-mouthed bottle and the moisture con-

tent made up to the optimum (two-thirds water-holding capacity). Two samples were incubated for one week and two for four weeks. The ammonia and nitrate nitrogen were then determined and reported as milligrams of nitrogen and nitrates ( $\text{NO}_3$ ), respectively, per 100 gm. of soil. In 1913 the ammonia was determined as follows: The water content was made up to a definite volume and the whole shaken for 45 minutes. Two gm. of calcium oxid were then added and the contents were again shaken for a short time and allowed to stand until the supernatant liquid became clear. A definite volume of this liquid was then distilled in the presence of magnesium oxid, the distillate being collected in standard acid and titrated. The calcium oxid was added as a clarifying agent in order to obtain a solution upon which nitrate and nitrite nitrogen could be determined colorimetrically. The presence of this reagent caused a perceptible increase in the ammonia set free, probably liberating some of the loosely attached nitrogen; but since the results are comparative and because of reasons already mentioned, the method seemed justifiable. However, such insignificant quantities of nitrate and nitrite nitrogen were found after seven days' incubation that these determinations were discontinued during 1914, and the ammonia was determined by direct distillation of the soil in copper flasks.

Where the incubation lasted for four weeks, the water loss by evaporation from the soil was replaced from time to time. Besides these experiments, samples were also run during 1914 with the addition of calcium carbonate in excess of that required to neutralize all nitric acid that could be formed from the cottonseed meal. Nitrate nitrogen was determined in all cases upon an aliquot part of a solution, obtained as directed above, using the phenoldisulphonic-acid colorimetric method.

The nitrifying inoculation experiments were conducted as follows: A soil possessing both a high nitrifying capacity and nitrifying efficiency, in the sense that Stevens and Withers (17) use these terms, was selected as a standard medium. To 100-gm. samples of this soil, cottonseed meal containing 60 mgm. of nitrogen was added, sufficient water added to bring it up to optimum, less 20 c. c., and the whole subjected to 20 pounds' pressure for one hour in the autoclave. These samples were then inoculated from the various plots with 20 c. c. of a soil suspension made by shaking 1 part of soil in 2 parts of water. The incubation covered a period of 28 days at room temperature. Ammonia and nitrates were determined as stated above. The results are reported in milligrams of nitrogen and nitrate ( $\text{NO}_3$ ), respectively, per 100 gm. of soil. It will be noted that the nitrate data for 1913 are low, in many places zero. This was caused by the failure to add calcium carbonate which was added in 1914. Apparently some substance toxic to nitrification is produced by heating. This substance gradually disappears on standing, and the disappearance is materially hastened by the addition of calcium carbonate.

The cross-inoculation experiments were tested by taking a mixture of all samples collected during both seasons from the respective plots, thoroughly mixing, and using as a medium 100-gm. samples containing 60 mgm. of nitrogen. These samples were also subjected to 20 pounds' pressure for one hour in the autoclave. A sufficient number of samples were thus prepared for duplicate inoculation from each plot under study. One of the duplicates received calcium carbonate; the other did not. These samples were incubated for six instead of four weeks and nitrate nitrogen determined as before.

In all the above-outlined experiments duplicate samples were set up and analyzed. Where possible, as in the nitrate determinations, duplicate determinations were run with each sample. If these varied widely, they were again run; or where this was impossible, they were discarded. In general, the duplicates agreed very well, except in the nitrifying inoculating experiments when incubated for only four weeks with no calcium carbonate added. Perhaps these results should not be included in the tabulated data; but since the relative positions of the averages do not materially differ from those of 1914, they have been included.

EXPERIMENTAL WORK

NUMBER OF BACTERIA

Table II gives the moisture content of soil from the different plots at the various samplings.

TABLE II.—Percentage of moisture in soil of the fertility plots when sampled <sup>a</sup>

Plot No.	1913						1914					
	July 11.	Aug. 12.	Sept. 12.	Nov. 4.	Dec. 20.	Average.	May 23.	June 22.	July 25.	Sept. 15.	Oct. 30.	Average.
1.....	15.5	5.0	6.0	20.0	21.3	13.56	7.6	7.6	10.6	20.8	19.5	13.20
13.....	16.0	4.0	4.8	18.0	20.9	12.75	8.0	6.5	7.6	21.0	20.6	12.74
3.....	.....	.....	.....	.....	.....	.....	7.0	6.0	9.0	20.8	20.0	12.56
10.....	18.0	14.2	12.0	17.5	24.3	17.20	10.0	9.0	17.0	22.2	20.0	15.64
9.....	.....	.....	.....	.....	.....	.....	8.0	7.4	12.0	21.6	18.8	13.56
2.....	.....	.....	.....	.....	.....	.....	8.0	8.1	12.0	20.0	17.8	13.20
18.....	15.0	7.6	7.0	19.5	20.4	13.0	14.0	14.7	10.0	21.0	20.0	15.94
17.....	16.0	6.8	7.4	18.4	21.0	13.92	13.0	14.7	8.6	17.1	18.0	14.28
22.....	16.0	5.8	6.2	17.4	21.2	13.32	8.4	9.4	15.0	22.2	22.8	15.56
23.....	16.0	6.2	7.0	19.0	21.5	13.90	11.2	7.4	14.8	20.4	21.6	15.08

<sup>a</sup> The soil was dried at 110° C. for two hours.

Table III gives the total number of bacterial colonies developing on Temple's agar expressed in millions per gram of dry soil. More emphasis should be placed upon the 1914 series than the 1913, because of the larger number of analyses and the greater uniformity of plots. A study of this table brings out several interesting facts, the most evident being that of the effect of manure. In all cases except the rotated plot for 1913, all those receiving manure rank materially higher than those not receiving it. Since this exception did not hold true for 1914, it is

possible that some other factor was influencing plot 20. The plots receiving chemical fertilizers ranked a little lower than the lowest receiving manure but materially higher than those receiving nothing.

TABLE III.—Number of bacteria per gram of soil <sup>a</sup>

[1,000 omitted]

Plot No.	1913					1914					
	July 11.	Aug. 12.	Nov. 4.	Dec. 20.	Average.	May 23.	June 22.	July 25.	Sept. 15.	Oct. 30.	Average.
1.....	7,800	4,000	.....	1,270	4,560	5,000	4,500	4,800	8,100	7,000	5,880
13.....	3,690	7,080	4,590	1,520	4,210	2,820	2,300	4,400	4,000	5,560	3,820
3.....	.....	.....	.....	.....	.....	4,800	3,000	5,000	6,000	7,500	5,270
10.....	10,730	9,900	.....	6,270	8,960	7,050	4,850	11,800	15,000	26,000	13,050
9.....	.....	.....	.....	.....	.....	1,840	1,800	3,500	4,300	3,450	2,980
2.....	.....	.....	.....	.....	.....	4,730	3,150	6,480	4,560	8,100	5,400
18.....	4,600	8,000	14,000	4,820	7,855	10,400	15,400	9,000	14,750	17,100	13,330
17.....	2,950	3,860	3,400	1,670	2,970	2,700	3,000	2,650	3,100	4,400	3,190
22.....	10,510	3,610	5,020	2,300	5,660	3,710	4,560	7,600	10,260	16,200	8,470
23.....	4,520	3,160	5,430	920	3,500	2,800	3,300	4,650	4,500	7,000	4,450

<sup>a</sup> The soil was dried at 110° C. for two hours.

If the effect of the various cropping systems in the absence of manure is considered, the various systems rank in the following order in 1914: (1) Timothy, (2) rotation, (3) corn, and (4) wheat. Wheat and corn are about equal, with a marked increase in the rotated and the timothy soils. In the presence of manure the rank is almost the reverse: (1) Corn, (2) wheat, (3) timothy, and (4) rotation. Here, again, wheat and corn are approximately equal, with a marked falling off to timothy and a somewhat less decrease to the rotation.

Just why manure should have the effect of raising the bacterial content of wheat and corn from the lowest to the first rank is not known. On the other hand, just why it should have less effect upon plots with a normally higher count is equally not understood. The peculiar behavior of the rotation plot receiving manure is very striking, the bacterial numbers being only slightly affected. Particularly is this so when we remember that this rotation is composed of corn, oats, wheat, clover, and timothy, since corn, wheat, and timothy bring about conditions which readily respond to manure. It should be remembered that if samples had been taken from rotation plots when some crop other than clover or timothy was growing, the results might have been different. A possible explanation of the lack of effect of manure upon the rotation plots and a less-marked effect upon timothy may lie in the amount of organic matter that these plots themselves return to the soil. The less the quantity of organic matter returned to the soil apparently the more marked is the result from manure.

The results secured from eight analyses of plots 29 and 30 (not given in Table III) indicate that the effect of manure is in part accumulative. Plot 29 had received only 6 applications of manure against 25 for plot 30,

while the average bacterial count for No. 29 was 7,840,000 and for No. 30 was 13,632,000. This accords with Temple's idea (20) that the increase in number is largely due to the fermentable material added and not to the bacteria actually carried in the manure.

#### FORMATION OF AMMONIA

The results presented in Table IV seem to the writers clearly to establish one of two facts, either that the systems of cropping under study exert no appreciable influence on the ammonia-forming power of this soil type or that the methods used for determining such differences are valueless. Since many investigators have been able to detect marked differences with essentially the same methods, the former conclusion might seem most likely. We would call special attention, however, to the results obtained by Given and Willis (6, 7) and Perotti (15), together with their conclusions regarding the existence of differences and the value of methods in vogue for determining this phenomenon. It is true that during 1913 the plots receiving manure gave slightly higher results than those receiving no such treatment, but it is equally true that the reverse is evident for 1914. Furthermore, there seems to be no correlation between the number of bacteria and the amount of ammonia formed.

It may be argued, in view of the nitrification data given later, that in the plots receiving manure the ammonia formed was transformed into nitrates. But, as previously mentioned, the accumulation of nitrates in seven days' time in the presence of cottonseed meal is practically nil. In fact, the senior writer (5) has demonstrated that under such conditions there is a rapid disappearance of nitrate nitrogen during the first few days. To ascertain this condition, determinations of nitrate nitrogen were made during 1913 which showed, as may be noted, that the above contention was verified. The greatest quantity of nitrate nitrogen recovered after one week's incubation was 3.5 mgm. from plot 10. It is true that there were large differences in the amount of ammonia in the four-week analyses; but if we add the nitrate and ammonia nitrogen, the differences are slight. From the ammonia figures it is also evident that in no case, even after four weeks' incubation, in the absence of calcium carbonate was nitrification limited by the formation of ammonia. This possibly did not hold true in a few cases when calcium carbonate was added.

#### FORMATION OF NITRATE

In our study of nitrate formation we have obtained the most marked and consistent results of any of our studies. This is graphically shown in figure 1 for the four-week incubated samples without calcium carbonate. Tables IV and V give the tabulated results of experiments conducted on nitrate formation in natural soil from different plots.

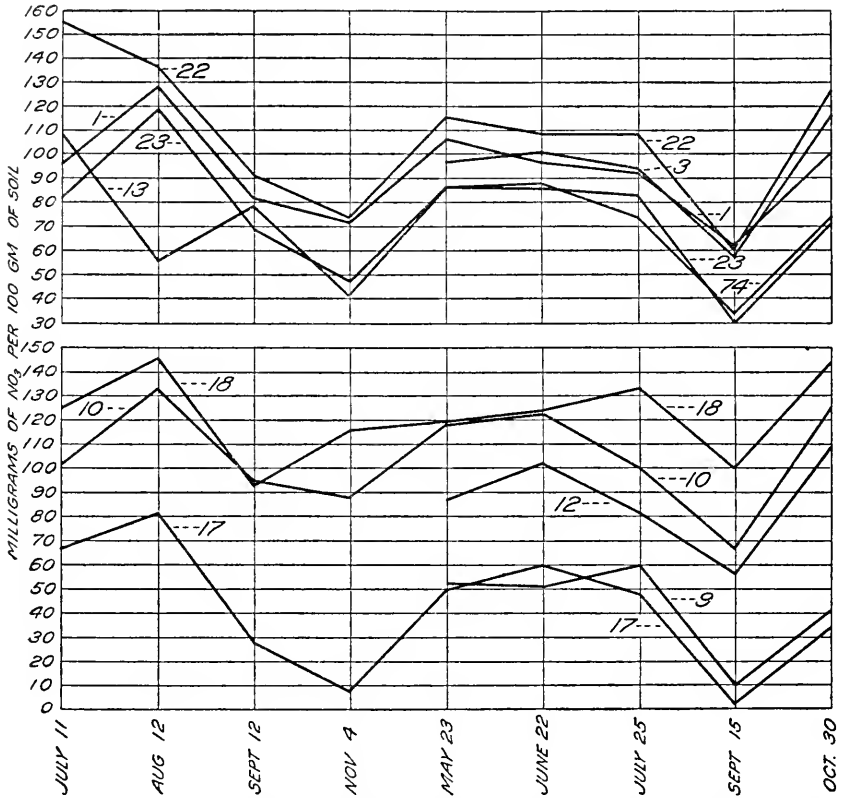


FIG. 1.—Curves of the nitrate formation in soil of fertility plots at Columbia, Mo., in 1913-14, after 28 days' incubation with the addition of 60 mgm. of nitrogen as cottonseed meal, but without the addition of calcium carbonate.

TABLE IV.—Ammonia and nitrate formation (in milligrams) in soil from different fertility plots at Columbia, Mo., during 7 days' incubation without addition of calcium carbonate

Plot No.	1913												1914					
	Nitrate (NO <sub>3</sub> ) recovered.				Nitrogen as ammonia recovered.				Nitrogen as nitrate and ammonia recovered.				Nitrogen as ammonia recovered.					
	Aug. 12.	Sept. 12.	Nov. 4.	Average.	Aug. 12.	Sept. 12.	Nov. 4.	Average.	Aug. 12.	Sept. 12.	Nov. 4.	Average.	May 23.	June 22.	July 25.	Sept. 15.	Oct. 30.	Average.
1.....	3.76	8.95	T. <sup>a</sup>	4.24	39.67	27.87	31.63	33.06	40.52	29.88	31.63	34.01	27.97	26.82	24.94	20.31	24.68	24.94
13.....	T. <sup>a</sup>	2.43	o	.80	40.35	29.14	32.70	34.06	40.35	29.69	32.70	34.24	28.31	29.71	26.88	24.00	26.99	27.18
3.....	o	o	o	o	o	o	o	o	o	o	o	o	28.43	27.93	25.79	20.50	25.41	25.61
10.....	4.30	15.78	T. <sup>a</sup>	6.69	46.27	31.67	34.23	37.37	47.23	35.22	34.23	38.89	21.84	25.31	24.52	21.70	24.03	23.48
9.....	o	o	o	o	o	o	o	o	o	o	o	o	28.10	27.93	26.22	21.70	25.90	25.99
2.....	o	o	o	o	o	o	o	o	o	o	o	o	27.88	28.07	27.63	21.70	28.81	26.94
18.....	6.69	5.09	T. <sup>a</sup>	3.93	37.20	31.25	36.53	34.99	38.70	32.40	36.53	35.87	21.65	24.40	24.52	19.64	24.44	22.93
17.....	o	1.41	o	.47	38.70	27.87	30.58	32.37	38.70	28.19	30.55	32.48	28.40	27.00	28.22	16.74	27.42	25.55
22.....	3.78	6.38	o	3.39	42.52	25.76	31.88	33.38	43.32	27.19	31.85	34.14	26.62	28.07	25.85	23.39	25.83	26.07
23.....	o	4.06	o	1.35	43.15	25.33	29.03	32.50	43.15	26.23	29.03	32.80	26.93	28.30	25.61	22.19	24.32	25.47

<sup>a</sup> T.—trace.



TABLE V.—Ammonia and nitrate formation (in milligrams) in soil from different fertility plots at Columbia, Mo., during 28 days' incubation with and without addition of calcium carbonate

Plot No.	1913 (without calcium carbonate).												1914 (without calcium carbonate).						1914 (with calcium carbonate).								
	Nitrate (NO <sub>3</sub> ) recovered.			Nitrogen as ammonia recovered.			Nitrogen as nitrate and ammonia recovered.			Nitrate (NO <sub>3</sub> ) recovered.			Nitrate (NO <sub>3</sub> ) recovered.			Nitrate (NO <sub>3</sub> ) recovered.			Nitrate (NO <sub>3</sub> ) recovered.								
	July 11.	Aug. 12.	Sept. 12.	Nov. 4.	Average.	July 11.	Aug. 12.	Sept. 12.	Nov. 4.	Average.	May 23.	June 22.	July 25.	Sept. 15.	Oct. 30.	Average.	May 23.	June 22.	July 25.	Sept. 15.	Oct. 30.	Average.					
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.				
1	95.8	128.0	0	61.7	9.49	24.71	18.78	31.86	21.22	31.16	53.69	37.42	48.28	45.89	105.8	57.3	92.3	62.0	100.0	91.5	141.0	133.3	144.0	146.0	175.9		
13	109.4	56.7	79.2	43.4	71.9	10.41	29.15	28.40	38.14	26.53	35.40	41.97	46.31	47.73	45.78	86.4	87.8	75.0	34.2	74.2	71.5	101.5	153.3	138.6	58.8	103.6	
3	101.1	133.0	95.6	88.3	104.5	8.35	21.01	19.41	22.77	18.14	31.22	37.79	41.03	42.74	41.09	120.0	124.1	100.0	67.9	124.1	107.2	104.4	147.0	150.0	120.0	163.5	
10	101.1	133.0	95.6	88.3	104.5	8.35	21.01	19.41	22.77	18.14	31.22	37.79	41.03	42.74	41.09	120.0	124.1	100.0	67.9	124.1	107.2	104.4	147.0	150.0	120.0	163.5	
9	101.1	133.0	95.6	88.3	104.5	8.35	21.01	19.41	22.77	18.14	31.22	37.79	41.03	42.74	41.09	120.0	124.1	100.0	67.9	124.1	107.2	104.4	147.0	150.0	120.0	163.5	
2	101.1	133.0	95.6	88.3	104.5	8.35	21.01	19.41	22.77	18.14	31.22	37.79	41.03	42.74	41.09	120.0	124.1	100.0	67.9	124.1	107.2	104.4	147.0	150.0	120.0	163.5	
18	125.2	146.3	91.7	110.2	130.2	6.14	6.02	17.52	21.03	12.09	34.16	39.05	38.69	47.00	40.00	87.8	102.8	81.8	56.3	109.0	87.5	144.0	153.2	144.0	73.4	158.3	
17	67.5	82.5	28.0	7.6	46.3	13.77	41.00	40.51	46.36	36.63	34.00	39.72	36.63	36.63	50.8	120.0	124.1	100.0	67.9	124.1	107.2	104.4	147.0	150.0	120.0	163.5	
22	155.8	137.3	91.2	71.2	114.6	7.87	29.43	14.62	31.24	19.63	31.61	33.46	35.25	47.95	40.00	69.6	120.0	124.1	100.0	67.9	124.1	107.2	104.4	147.0	150.0	120.0	163.5
21	81.7	119.2	69.3	47.4	79.4	11.77	33.04	29.91	39.66	28.59	30.25	39.00	45.70	50.38	46.33	85.7	135.7	62.7	30.0	71.1	71.0	147.0	153.2	150.0	100.0	107.4	

Here, again, the marked effect of manure, particularly in the case of corn and wheat, is evident. These two plots rank at the bottom of the series when untreated, but by the addition of manure they are moved from ninth and tenth places to first and second, manure having a much greater influence on these than on any other plots studied. The effect of manure is quite marked on the timothy and rotation plots, though not nearly so great as in the case of corn and wheat. Considering both seasons, we find the percentage of increase caused by the application of manure to be as follows:

	1914	1913
Corn.....per cent..	217	160
Wheat.....per cent..	150	...
Timothy.....per cent..	47	44
Rotation.....per cent..	28	32

The increases due to the application of chemicals were as follows: Wheat, 103 per cent; rotation, 30 per cent.

These figures are from the average of those samples receiving no calcium carbonate. Considering the same phenomenon when calcium carbonate was added to the test samples, we find entirely different data: Corn, 39 per cent; wheat, 21 per cent; timothy, no increase; rotation, 9 per cent. When chemicals were added the increase of wheat was 9 per cent; that of the rotation plot, 4 per cent.

The application of calcium carbonate to the soil from different plots in 1914 gives some equally interesting data:

Plot No.	Cropping system.	Percentage of increase due to calcium carbonate.
17.....	Continuous corn.....	162
18.....	Continuous corn, with the addition of manure.....	15
9.....	Continuous wheat.....	187
10.....	Continuous wheat, with the addition of manure.....	40
2.....	Continuous wheat, with the addition of chemicals.....	54
23.....	Continuous timothy.....	102
22.....	Continuous timothy, with the addition of manure.....	37
13.....	Six-year rotation.....	89
1.....	Six-year rotation, with the addition of manure.....	61
3.....	Six-year rotation, with the addition of chemicals.....	50

Calculating from the 1914 tests the percentage increase in the nitrate formation of manured soil with the addition of calcium carbonate over the untreated in the absence of calcium carbonate, we approximate the combined influence of both factors. Estimating the theoretical effect of the two factors by adding the increases resulting from manure alone and from calcium carbonate alone, very different results are obtained.

From these data and those given above it is evident that in only one case of the series studied is it possible to replace entirely the calcium carbonate by manure or the manure by calcium carbonate. However, this may be done to a very large extent. In the case of timothy it is not

possible to replace calcium carbonate with manure. The effect of manure, however, can be entirely eliminated by calcium carbonate. As perhaps would be expected, the percentage increases due to calcium carbonate not replaceable by manure are in an inverse ratio to those of manure not replaceable by calcium carbonate. Such increases seem to be, in general, correlated with the type of crop—that is, those crops naturally depleting the soil of organic matter (corn and wheat) show a large percentage increase from manure not replaceable by calcium carbonate, while those naturally keeping up the organic matter (rotated) show a larger percentage due to calcium carbonate not replaceable by manure.

Cropping system.	Percentage of increase due to manure and calcium carbonate.		Difference.
	Actual.	Calculated.	
Corn.....	264	379	115
Wheat.....	247	337	90
Timothy.....	102	149	47
Rotation.....	107	117	10

It seems evident that in a general way the effect of the two agencies are the same, so far as nitrate formation is concerned. This does not support Temple's contention (20) that the beneficial effect of manure is due to organisms actually brought in with the manure.

Cropping system.	Percentage of increase due to calcium carbonate in presence of manure or not replaceable by it.	Percentage of increase due to manure in presence of calcium carbonate or not replaceable by it.
Corn.....	15	39
Wheat.....	40	21
Timothy.....	37	0
Rotation.....	61	9

It is also interesting to note that in the rotation studied either with or without the addition of calcium carbonate, the effect on nitrate formation of the chemicals has been practically identical with that of the manure. In the case of wheat, chemicals have had only about one-half of the beneficial effect that manure has had. As to the effects of the different crops, corn and wheat undoubtedly have a harmful effect in the absence of manure, both ranking very low either with or without calcium carbonate. On the other hand, with the addition of manure they are raised from tenth and ninth places to first and second places, respectively. Timothy and the rotation are approximately equal and very much higher than corn

and wheat. The effect of manure, however, was not very marked on either, being somewhat more pronounced in the case of timothy. When calcium carbonate was added to the test samples the only noticeable effect of the crop is the low position held by corn and wheat in absence of manure.

The fact that the addition of calcium carbonate to samples eliminates to a large extent the very large and unmistakable differences, otherwise detectable, raises the question as to which method probably more accurately represents field conditions. We shall only call attention to the fact that Löhns and Green (11) vigorously maintain that the addition of calcium carbonate is essential, while Temple (21) has shown that with organic sources of nitrogen vigorous nitrification is possible even in acid soils. Table VI gives the results of an experiment to determine the effect of varying the quantity of nitrogen and calcium carbonate added. This test was run in order to determine the specific quantity of calcium carbonate necessary to insure maximum nitrification and also the correct amount of nitrogen to be added.

TABLE VI.—Effect on nitrate formation of varying the quantity of calcium carbonate and nitrogen added to soil

Plot 17.					Plot 18.				
Calcium carbonate.	Nitrogen as cotton-seed meal.	Nitrate.	Nitrogen as ammonia.	Nitrate.	Calcium carbonate.	Nitrogen as cotton-seed meal.	Nitrate.	Nitrogen as ammonia.	Nitrate.
Gm.	Mgm.	Mgm.	Mgm.	Mgm.	Gm.	Mgm.	Mgm.	Mgm.	Mgm.
a 0.....	60	60	60	13.7	a 0.....	60	104.3	60	43.2
a .05.....	60	75.8	60	23.4	a .05.....	60	133.3	60	66.4
a .10.....	60	82.7	60	34.2	a .10.....	60	124.7	60	85.2
a .25.....	60	94.2	60	42.0	a .25.....	60	135.8	60	156.5
a .50.....	60	146.9	60	55.3	a .50.....	60	144.0	60	218.1
a 1.00.....	60	141.9	60	74.0	a 1.00.....	60	138.4	60	232.2
a 2.50.....	60	144.0	60	70.8	a 2.50.....	60	162.8	60	244.0
a 5.00.....	60	144.0	60	80.0	a 5.00.....	60	135.8	60	244.0
b 1.....	0	22.3	0	28.2	b 1.....	0	32.5	0	31.5
b 1.....	15	56.8	15	60.0	b 1.....	15	58.3	15	80.0
b 1.....	30	85.7	30	75.0	b 1.....	30	92.3	30	108.2
b 1.....	60	144.0	60	90.0	b 1.....	60	142.8	60	266.6
b 1.....	120	193.5	120	37.2	b 1.....	120	244.0	120	369.0
b 1.....	240	8.8	240	7.8	b 1.....	240	444.4	240	232.2
c 0.....	0	14.0	0	14.0	c 0.....	0	20.0	0	20.0

<sup>a</sup> Nitrogen constant, calcium carbonate varying.

<sup>b</sup> Calcium carbonate constant, nitrogen varying.

<sup>c</sup> Nitrate originally in soil.

The amount of nitrate formed in seven days is so insignificant that we have left the 7-day data out of consideration. It is interesting, however, to note that those plots which rank high for 28 days also rank high for 7 days.

A study of the relative position of the different plots at the various analyses shows clearly that the method used is reliable for detecting

differences, whether the nitrate-forming power is low or high. Table VII gives the relative rank of the different plots at each analysis. Figure 1 illustrates very clearly the low and the high nitrate-forming power at different periods.

TABLE VII.—Average nitrate formation without addition of calcium carbonate (Table V) of seven fertility plots studied in 1913 and 1914, together with their relative rank at each sampling

Plot No.	1913				1914						
	Average nitrate formation.	Rank.				Average nitrate formation.	Rank.				
		July 11.	Aug. 12.	Sept. 12.	Nov. 4.		May 23.	June 22.	July 25.	Sept. 15.	Oct. 30.
	<i>Mgm.</i>					<i>Mgm.</i>					
1.....	94.7	5	4	4	4	91.5	4	4	4	3	4
10.....	104.5	4	2	1	2	107.2	2	2	3	2	3
13.....	71.9	3	7	5	6	71.5	5	5	6	5	5
17.....	46.4	7	6	7	7	39.2	7	7	7	7	7
18.....	120.2	2	1	2	1	124.2	1	1	1	1	1
22.....	114.6	1	3	3	3	104.4	3	3	2	4	2
23.....	79.4	6	5	6	5	71.0	6	6	5	6	6

Since the addition of calcium carbonate has to such a large extent eliminated the differences in nitrate formation when testing in the soil itself, it is interesting to ascertain whether this can be traced to an elimination of acid conditions. Dr. P. F. Trowbridge, of the Department of Agricultural Chemistry, Missouri Experiment Station, has furnished us with the following data regarding the lime requirements of some of the plots. The figures are in pounds per acre-foot; basis, 3,000,000 pounds per acre: Plot 2, 7,900; plot 13, 7,200; plot 17, 7,900; plot 18, 2,400; plot 22, 2,400; plot 23, 7,200 pounds.

It will be noted that, so far as the crop is concerned, the differences in lime requirements are very slight (plots 13, 17, and 23 or 18 and 22). Manure kept the acidity low (plots 18 and 22). Commercial fertilizers have had no appreciable effect upon acidity (plot 2). Nevertheless, continuous corn soil with a lime requirement not materially different from that of timothy, the rotation, or continuous wheat receiving commercial fertilizers produced only about one-half the amount of nitrates as these soils. It will be shown later that transferring the organisms to a common pabulum containing an abundance of calcium carbonate does not eliminate the large differences. In this connection, as a suggested explanation of one of the effects of lime when added to the soil, we call attention to the work of Schreiner and Reed (16), who have demonstrated the stimulative effect of calcium carbonate upon oxidases. It is not impossible that soil conditions are sometimes such as to permit the accumulation of nitrate-forming oxidases, while other conditions

will not permit similar accumulations. The work of the Bureau of Soils has also demonstrated that calcium carbonate is rather efficient in eliminating the toxic effect of certain decomposition products found in soils, and the beneficial effect might, in part, be due to an action of this nature.

The following data were obtained during 1913 concerning the relative effect of 6 years' application of manure compared with that for 25 years: Plot 29, continuous wheat, received manure for 6 years and produced in one week's incubation an average of 5.35 mgm. of nitrate; in four weeks an average of 116.6 mgm. In the inoculation experiments it produced 15.3 mgm. Plot 30 received manure for 25 years, otherwise similar to No. 29, and produced in one week 6.5 mgm. of nitrate; in four weeks 127 mgm., and in inoculating experiments, 27.5 mgm. This indicates that the shorter period of application has produced almost the same effect as the longer.

#### NITRIFYING INOCULATION EXPERIMENTS

The results of the nitrifying inoculation experiments are reported in Table VIII. It should be borne in mind that the 1913 results were obtained under conditions not favorable for nitrate formation, no calcium carbonate having been added. Therefore, too much weight should not be attached to these results. The 1914 results, however, were secured under favorable conditions. It is worthy of note, though, that there is a very close agreement between the relative rank of the plots in the inoculating experiments for both seasons and the nitrate-forming experiments. This is shown in Table IX, together with the relative position of bacterial numbers.

The agreement is not absolute, but it is close enough to indicate the probability of the same factors controlling the nitrate formation in the two instances. This being true, the factors must be biological in nature rather than chemical or physical; otherwise they probably would not be transferred in 20 c. c. of a 2 to 1 soil suspension in sufficient quantities to exercise much influence. There is also exhibited here a close correlation between bacterial numbers and nitrate formation. Since, however, no such correlation can be traced in ammonia formation, there is little reason for believing the two factors connected other than that probably the factors controlling both are the same.

TABLE VIII.—Ammonia and nitrate formation (in milligrams) in soil X when inoculated with soil from different fertility plots during 28 days' incubation.

Inoculum plot No.	1913											1914				
	Nitrate (NO <sub>3</sub> ) recovered.					Nitrogen as ammonia recovered.					Nitrogen as nitrate and ammonia recovered.					
	Aug. 12.	Sept. 12.	Nov. 4.	Dec. 20.	Average.	Aug. 12.	Sept. 12.	Nov. 4.	Dec. 20.	Average.	May 23.	June 22.	July 25.	Sept. 15.	Oct. 30.	Average.
1.	34.2	31.6	0	0	16.4	37.02	35.66	47.68	29.96	41.30	125.2	54.0	41.6	54.0	70.5	69.1
3.	5.7	32.6	0	0	9.6	48.81	33.25	49.19	33.01	43.17	65.4	10.3	14.3	20.3	25.7	27.2
10.	10.2	14.7	7.0	2.0	10.0	49.63	30.57	47.03	28.72	38.99	180.0	53.3	63.1	78.2	96.1	81.7
2.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	37.1	5.0	7.5	7.4	5.2	12.4
9.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	156.6	83.0	55.5	34.8	44.4	74.9
18.	70.7	55.1	4.7	3.3	35.0	28.40	20.74	37.93	29.96	36.70	180.0	82.7	58.2	64.3	161.1	109.3
17.	.....	.....	.....	.....	.....	48.36	36.02	47.68	26.28	39.58	80.0	9.8	5.3	Trace.	6.3	20.3
22.	23.3	38.2	0	3.2	16.2	44.38	28.02	44.64	24.15	35.37	141.7	35.0	46.1	38.5	60.6	66.2
23.	4.0	7.0	0	0	2.7	52.29	32.69	46.00	19.92	37.82	93.0	10.6	33.3	20.2	8.6	33.1

TABLE IX.—Comparison of the ranks in 1913 and 1914 of the bacterial numbers and nitrate-forming and inoculation experiments

Plot No.	1913			1914		
	Bacterial numbers.	Nitrate-forming experiments.	Inoculation experiments.	Bacterial numbers.	Nitrate-forming experiments.	Inoculation experiments.
1 . . . .	4	4	2	4	4	3
13 . . .	5	6	5	6	5	6
10 . . .	1	3	4	2	2	2
18 . . .	1	1	1	1	1	1
17 . . .	7	7	6	7	7	7
22 . . .	3	2	3	3	3	4
23 . . .	6	5	7	6	6	5

## CROSS-INOCULATION EXPERIMENTS

Unfortunately, time permitted the conducting of only one experiment along the line of cross-inoculation. An effort was made to make this as representative as possible, both by using as the pabulum a mixture of all samples collected from the respective plots during both seasons and by inoculating in duplicate sterile samples, containing 60 mgm. of nitrogen as cottonseed meal from every other plot. In addition, calcium carbonate was added to one of the duplicates. The results are reported in Table X.

Reading horizontally, one obtains figures representing the capacity—using this term in the sense that Stevens and Withers (17) used it—of soil from the different plots to support nitrification both with and without the addition of calcium carbonate. It is noted that all the soils will support vigorous nitrification, provided vigorous nitrifiers are added. If the averages in the last column are examined, it will be noted that when calcium carbonate is added differences can be noted, but they are not so marked as some other results, the highest figure being 99.9 and the lowest 72.7. In the absence of calcium carbonate the figures are not nearly so high, and, though the differences are somewhat more marked, the relative positions are not materially different from those where calcium carbonate was added. Examining any vertical column, we obtain figures representing the ability of the various soils to support the nitrifying flora from any one particular soil; or inversely, the ability of any particular soil to inoculate the others. This also varies materially, indicating that the soil does exercise a marked influence upon nitrification, some floras thriving better in one soil and other floras better in others. Considering the average of the vertical columns, we obtain what may be termed the “relative inoculating ability.” Here we obtain our greatest variation, indicating, as previously suggested, that to the flora itself must be ascribed the major differences in nitrification. Here again, the continuously cropped plots, wheat and corn, rank lowest when no manure is added; but manure exercises a greater influence on them than any other in the series. In the case of timothy, manure has not increased its inoculating ability; in fact, the reverse is true, while with the rotated plot the effect has not been nearly so great as with corn and wheat.



TABLE X.—Results of cross-inoculation soil experiments expressed in milligrams of nitrate per 100 gm. of soil

Plot No.	Nitrate (NO <sub>2</sub> ), with addition of calcium carbonate.										Nitrate (NO <sub>2</sub> ), without addition of calcium carbonate.											
											Aver- age.											
	1	2	3	9	10	13	17	18	22	23	1	2	3	9	10	13	17	18	22	23	Aver- age.	
1.....	72.0	108.0	108.0	0	180.0	86.4	24.0	112.5	36.6	83.7	81.1	36.0	33.5	8.3	0	45.9	12.2	0	35.4	0	12.6	18.4
13.....	116.1	100.0	.....	0	109.0	75.0	26.1	116.1	85.7	92.3	80.0	21.0	31.3	14.6	0	50.2	33.2	6.2	25.7	6.6	22.5	21.7
3.....	124.1	63.5	50.8	0	171.4	83.8	9.0	112.5	25.7	80.0	72.7	31.7	32.7	7.6	0	69.6	15.0	0	24.8	0	9.2	19.1
10.....	150.0	.....	128.5	4.0	180.0	94.7	17.4	112.5	109.0	69.2	96.2	60.0	.....	12.8	0	40.0	29.1	4.1	33.7	7.8	20.0	20.7
9.....	120.0	90.0	50.8	0	103.6	86.4	30.8	128.5	38.5	62.0	77.7	27.1	25.7	5.1	0	30.8	18.0	0	27.3	0	7.8	14.2
2.....	128.5	138.4	94.7	0	133.3	43.2	6.0	85.7	36.5	85.7	75.4	34.8	46.9	8.1	0	31.3	16.3	0	28.8	0	11.6	17.8
17.....	94.7	97.3	105.8	0	133.3	87.8	30.8	81.8	46.9	54.5	73.3	12.0	15.0	3.9	0	27.7	23.3	5.0	23.2	0	17.1	12.8
18.....	144.0	150.0	100.0	0	144.0	87.8	42.3	128.5	60.0	94.7	92.1	.....	65.4	15.6	0	75.0	42.3	13.3	63.1	4.8	27.6	34.1
22.....	128.5	133.3	109.0	0	150.0	150.0	40.9	144.0	21.2	94.7	97.8	25.7	50.8	17.1	0	76.5	45.9	9.7	76.5	5.3	30.4	34.1
23.....	150.5	86.4	144.0	0	180.0	138.4	45.0	150.0	52.0	45.9	100.8	43.2	28.4	12.4	0	76.5	50.2	7.2	54.0	0	38.2	29.7
Average.....	137.1	104.1	100.4	0.4	154.4	93.3	27.8	117.2	51.5	76.3	.....	32.4	37.0	10.5	0	52.9	28.5	4.5	39.3	2.4	18.7	.....

The nitrifying capacity averages in the last column of Table X place timothy at the top of the list, manure in this case having no effect. Next in order are wheat and corn, where manure is added. Manure had no increasing effect on the rotated plot. The continuously cropped plots without manure, the rotated plots, and the plots receiving chemicals vary little. Manure here exercises little or no influence on those crops not depleting the soil of organic matter.

It is evident that the nitrifying floras of plots 9, 17, and 22, the first two especially, were extremely weak. Though these plots possessed at this time good nitrate-forming floras, they were not able to overcome the adverse effects experienced in transferring them to soils slightly less favorable but in which a more vigorous or differently constituted flora thrived.

#### NITRATE NITROGEN UNDER FIELD CONDITIONS

In Table XI is given the quantity of nitrogen as nitrate per 100 gm. of soil when the soil was collected from the field, but nothing particularly marked is to be noted from the results given. As would be expected, this quantity varies considerably; but since the demands made upon the various plots by the growing crop differ widely, little information is furnished regarding the rate of formation. After the wheat is harvested the nitrate content of wheat plots increases rapidly. Wheat was cut on June 20, 1913, and on June 28, 1914. Some accumulation is evident even before harvest. When wheat is growing rapidly no nitrates are present. The accumulation is much more marked in the presence of manure and chemicals than in their absence. There is an abundance of nitrate nitrogen under corn even when it is making its most rapid growth. During 1913 the water content fell so low in July and August that no nitrates could be formed; with rain coming in October, however, the nitrate content rose rapidly. In all cases where a comparison of the same crop in the presence and absence of manure is possible the nitrate content of the manure plot is materially higher than that of the unmanured.

TABLE XI.—Quantity (in milligrams per 100 grams of soil) of nitrate in soil when sampled from fertility plots at Columbia, Mo., in 1913 and 1914<sup>a</sup>

Plot No.	Quantity of nitrate (NO <sub>3</sub> ).											
	1913						1914					
	July 11.	Aug. 12.	Sept. 12.	Nov. 4.	Dec. 20.	Aver- age.	May 23.	June 22.	July 25.	Sept. 15.	Oct. 30.	Aver- age.
1.....	3.91	2.06	10.66	7.71	2.72	5.41	1.94	3.20	4.00	1.50	1.60	2.45
13.....	1.43	.75	1.07	1.90	1.25	1.28	2.10	1.13	2.10	0.75	Trace	1.22
3.....							0	1.27	2.00	1.20	1.50	1.19
10.....	6.40	8.78	18.00	11.08	Trace.	8.85	1.97	3.60	8.50	6.40	5.00	5.09
9.....							0	1.71	5.50	4.50	3.00	2.94
2.....							0	2.70	4.80	4.50	7.50	3.90
18.....	4.48	.75	4.46	5.67	3.57	3.79	2.40	6.00	12.20	7.20	2.50	6.06
17.....	2.48	.66	.53	2.10	3.06	1.76	3.10	6.12	6.43	1.70	1.90	3.85
22.....	1.94	1.91	6.50	1.90	Trace.	2.45	2.10	1.80	3.60	5.40	3.10	3.20
23.....	1.38	1.28	2.73	Trace.	Trace.	1.08	0	0	1.80	2.90	1.20	1.18

<sup>a</sup> Soil was dried at 110° C. for two hours.

## SUMMARY

(1) The agricultural methods practiced upon the plots under study have brought about marked differences in the number of organisms contained in the soil, at least those capable of developing under our experimental conditions. The soil under continuous corn and wheat contains, in the absence of any additions of fertilizers or manure, relatively low numbers of bacteria. In the presence of manure, continuous corn and wheat soil contain relatively high numbers, manure having a much more marked effect upon numbers here than under the other crops studied.

(2) The agricultural practices under study have, so far as we can detect without experimental methods, produced no appreciable effect upon the ability of the soil and its organic life to liberate ammonia from cottonseed meal.

(3) The ability of the soil complex to oxidize ammonia nitrogen to nitrate nitrogen has been materially altered by the methods under study. This we believe to be due in part to physical and chemical changes in the soil and in part to biological changes. Continuous corn and wheat with no additions of manure or chemicals have brought about a relative low oxidizing power in the soil complex. The addition of manure materially raises the oxidizing power, especially under continuous corn and wheat. The addition of commercial fertilizer brings about a condition similar to that of manure, though perhaps less marked.

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## STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL.—XV. DWARF EGGS<sup>1</sup>

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### INTRODUCTION

Eggs much smaller than normal eggs are occasionally produced by domestic fowls of all breeds. These eggs usually contain little or no yolk; but occasionally a small yolk, usually without germ disk but inclosed in a complete vitelline membrane, is present. The albumen is small in amount, and often but not always it is of a thicker consistency than the albumen of a normal egg. The egg membranes are normal. The shell varies in thickness over the same range as the shells of normal eggs. Sometimes, as in eggs otherwise normal, shell is entirely lacking—that is, the egg is simply covered with a membrane.

These small eggs are called by various names as “cock eggs,” “witch eggs,” “luck eggs,” “wind eggs,” “dwarf eggs,” etc. Most of these names are associated with interesting superstitions. The term used by the people in any particular part of the world depends in part on the folklore of the region. Since no single term is generally accepted, we have decided to use a name which, although it has no legendary history, is somewhat descriptive. We have therefore called these small eggs “dwarf eggs.”

Among the various types of abnormal eggs produced by the domestic fowl the dwarf egg is more common than any other type except the double-yolked egg. This type of egg has played an important rôle in the folklore of all nations. Sebillot (22),<sup>2</sup> Tiedeman (25), König-Warthausen (7), and Bonnet (2) give some of the popular superstitions connected with these eggs. A widespread superstition which comes down nearly to our own time is that a cock, or especially a very old cock, produces these eggs. These “cock” eggs were sometimes supposed to be made up of semen and “humors.” A superstition which was quite widely accepted at an early period was that such an egg might hatch into a fabled

<sup>1</sup> Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 98.

<sup>2</sup> Reference is made by numbers to “Literature cited,” p. 1041.

serpent, the basilisk, whose breath or look was fatal. The basilisk was sometimes said to have head and legs like a cock. Less definite superstitions which simply regard the "cock" egg as an evil omen have also been common. In some places European peasants cast these eggs behind them over a wall or building to avoid bad luck. In other places they used them as evil charms to avenge themselves on their enemies. A very mild sort of vengeance practiced in some localities was to place one of these eggs among the eggs belonging to a neighbor. This prevented his eggs from hatching. A more violent charm was made by breaking the dwarf egg and filling part of the shell with dew collected at dawn from a white thorn tree and then exposing this to the sun. A terrible calamity was supposed to happen to the designated person as the sun drank the last drop of dew.

On the other hand, these eggs have been considered as a sign of good luck. Pearl (11) reported such a superstition which only a few years ago was accepted by a few credulous country people in some parts of the United States. According to this version of the myth a "luck egg" does not break when thrown over a building, and any wish made by the thrower while the egg is in the air is sure to come true.

The dwarf, witch, or cock egg has emerged from the age of superstition with the cause for its production inadequately explained. It is the purpose of the present paper to discuss (1) the different types of dwarf eggs in respect to shape and also in respect to contents; (2) the variability in respect to size and shape; (3) the interrelations of the variations in dimensions, shape, and size; (4) the frequency of the occurrence of dwarf eggs compared to normal eggs and of dwarf-egg producers compared to birds which do not lay dwarf eggs; (5) the seasonal distribution of dwarf eggs; (6) dwarf-egg production by birds with normal and with abnormal oviducts; (7) the relation of dwarf-egg production by normal birds to the age of the bird and to the position of the egg in the clutch and litter; (8) physiological conditions which lead to dwarf-egg production; (9) the relation of the production of dwarf eggs to other abnormal phenomena of reproduction which either occur in nature or have been experimentally produced; and (10) the contribution which the study of the physiology of dwarf-egg production makes to our knowledge of the normal physiology of egg production.

Since February, 1908, the abnormal eggs laid at the poultry plant of the Maine Agricultural Experiment Station have been brought to the laboratory for examination. In the eight years to February, 1916, 298 dwarf eggs are known to have been produced at this plant. The weight of 275 of these was taken, and in 261 of these cases the length and breadth were also measured and the length-breadth index calculated. Of the 298 eggs recorded 274 were opened, and their contents were examined. Several of the dwarf eggs were floor eggs and a few were laid by birds on which no egg record was kept. In 251 cases, however, the egg record

of the bird laying the dwarf egg is available. Furthermore, autopsies were made on several of these birds, and the condition of their sex organs was observed.

I.—CLASSIFICATION OF DIFFERENT TYPES OF DWARF EGGS, FIRST, IN RESPECT TO SHAPE, AND SECOND, IN RESPECT TO PRESENCE OR ABSENCE OF YOLK

The dwarf eggs of the fowl vary greatly in size and shape. Plate CXII shows 14 of these eggs with a normal egg laid by a 9-months-old pullet for comparison. From this illustration it may be seen that there are two distinct types of dwarf eggs in respect to their shape: The prolate-spheroidal type, similar in shape to a normal egg; and the cylindrical type, which is much longer in proportion to the breadth. The cylindrical eggs are shown in the first row of Plate CXII. These cylindrical eggs occur much less frequently than do the dwarf eggs of the prolate-spheroidal type. Of the 261 dwarf eggs on which complete data are available only 12, or 4.6 per cent, were of this form.

Not only do the dwarf eggs differ in respect to size and shape but there is a difference in internal structure. These dwarf eggs are sometimes defined as yolkless eggs, or small eggs containing a small quantity of yolk usually not in a yolk membrane. During this investigation the contents of 274 dwarf eggs were examined. It was found that some of these eggs contained no yolk but appeared to be formed around a nucleus which consisted of a few strings of coagulated albumen, apparently untwisted chalazal threads and also sometimes small lumps of hardened albumen or small blood clots. On the other hand, some contain small yolks in yolk membranes. These yolks do not usually have visible germ disks. The weight of these yolks varies from 1 gm. to nearly 8 gm. More than half of all the eggs opened, however, contained some yolk which was not inclosed in a yolk membrane. Dwarf eggs may then be classified according to the nonoccurrence of yolk and the condition of the yolk when present as, first, yolkless, second, with some yolk not in a membrane, and third, with one small yolk. In Table I the dwarf eggs are classified both according to form and yolk content.

TABLE I.—*Classification of dwarf eggs both as to shape and as to yolk content*

Shape.	Number yolkless.	Percentage yolkless.	Number with some yolk not in a membrane.	Percentage with some yolk not in a membrane.	Number with one small yolk.	Percentage with one small yolk.	Total.
Shape not known <sup>a</sup> ...	5	38.46	8	61.54	0	0	13
Prolate-spheroidal shape.....	83	33.33	139	55.82	27	10.84	249
Cylindrical shape....	8	66.67	4	33.33	0	0	12
Total.....	96	35.03	151	55.11	27	9.85	274

<sup>a</sup> Dimensions not recorded.

From the last line of Table I it is seen that 96, or 35.03 per cent, of the eggs opened were yolkless. The other 178, or 64.96 per cent, contained yolk. Of these, 151, or 55.11 per cent, of all the dwarf eggs opened contained yolk not inclosed in a yolk membrane. A small yolk was present in 27, or 9.85 per cent, of the dwarf eggs. From these figures it is seen that nearly two-thirds of the dwarf eggs contain yolk.

## II.—THE ALBUMEN AND SHELL OF DWARF EGGS

We have seen that dwarf eggs differ in respect to the nucleus around which the albumen is formed. Bonnet (3) states that the nature of the albumen is also generally altered. The dwarf eggs observed differed greatly in respect to the density of the albumen. In many it was very condensed, being a thick clear mass which nearly maintained its shape when removed from the shell and egg membranes. It appeared very much like the albumen in a normal egg while it is in the albumen-secreting region, or the isthmus of the oviduct (15). In many other cases it appeared exactly like the albumen of a normal laid egg—that is, there was a somewhat firm inner mass surrounded by a thin fluid albumen. All gradations between these also occurred. In a very few cases the albumen was more fluid than in the average normal egg. There was, however, an undoubted general tendency for the albumen to be more than normally firm. The density of the albumen was not determined accurately as a routine procedure in the dwarf eggs. Its apparent density as compared to normal eggs was frequently, but unfortunately not uniformly, recorded. In connection with another investigation in progress at this laboratory the specific gravity and refractive index of the albumen of many normal and a few dwarf eggs was determined. These probably were not a random sample of dwarf eggs. The minimum specific gravity of the sample of 7 dwarf eggs was 1.02824, while the mean specific gravity for the sample of 180 normal eggs was 1.0288. The dwarf eggs ranged widely, with the upper end of the range decidedly above that for normal eggs. The maximum for dwarf eggs was 1.2107, against a maximum of 1.0415 for the normal eggs. The mean for the dwarf eggs was 1.0627, which is higher than the maximum for normal eggs. The range, however, overlaps, 4 of the 7 dwarf eggs falling within the upper end of the range for normal eggs. In a sample of 10 dwarf eggs the refractive index lay within the range for the sample of 180 normal eggs. The mean was slightly higher for the dwarf than for the normal eggs; but this difference certainly was not significant.

The egg membranes of dwarf eggs, so far as superficial appearances indicate, are comparable to those of normal eggs. The shell is sometimes entirely or almost entirely absent, as in the case of membrane-covered or soft-shelled eggs, which are normal in all other particulars. The thickness of shell varies from very thin to very thick, as in normal eggs.

In the present investigation no further distinction is made between dwarf eggs in respect to variation in albumen or shell.

III.—SIZE AND SHAPE RELATIONS OF THE SEVERAL CLASSES OF DWARF EGGS COMPARED TO EACH OTHER AND TO NORMAL EGGS AND THE RELATIVE VARIABILITY OF NORMAL AND OF THE DIFFERENT CLASSES OF DWARF EGGS

There is a considerable amount of variation within each class of dwarf eggs in respect to every measurable character. Table II gives for each class the frequency distribution of variation for each dimension, the shape index, and the weight.

TABLE II.—Frequency distributions of the variation in size and shape of the several classes of dwarf eggs

Class.	LENGTH			Frequency of cylindrical shape.	
	Frequency of prolate-spheroidal shape.			Yolkless.	Some free yolk.
	Yolkless.	Some free yolks.	A small yolk.		
<i>Mm.</i>					
20. 0-22. 9.....	1	1			I
23. 0-25. 9.....	2	1			0
26. 0-28. 9.....	5	11		1	0
29. 0-31. 9.....	14	32		0	2
32. 0-34. 9.....	16	25	1	0	I
35. 0-37. 9.....	23	16	6	3	
38. 0-40. 9.....	11	23	4	1	
41. 0-43. 9.....	6	16	7	0	
44. 0-46. 9.....	5	7	7	1	
47. 0-49. 9.....		5	1	0	
50. 0-52. 9.....		1		1	
53. 0-55. 9.....				0	
56. 0-58. 9.....				1	
Total.....	83	<sup>a</sup> 138	<sup>a</sup> 26	8	4

BREADTH

<i>Mm.</i>					
6. 0-7. 9.....					I
8. 0-9. 9.....					0
10. 0-11. 9.....					0
12. 0-13. 9.....					0
14. 0-15. 9.....					I
16. 0-17. 9.....					I
18. 0-19. 9.....	1	1		1	0
20. 0-21. 9.....	3	3		1	0
22. 0-23. 9.....	7	5		3	I
24. 0-25. 9.....	7	18		0	
26. 0-27. 9.....	15	35	1	3	
28. 0-29. 9.....	23	25	6		
30. 0-31. 9.....	20	20	5		
32. 0-33. 9.....	6	18	5		
34. 0-35. 9.....	1	9	7		
36. 0-37. 9.....		4	1		
38. 0-39. 9.....			1		
Total.....	83	<sup>a</sup> 138	<sup>a</sup> 26	8	4

<sup>a</sup> Two dwarf eggs of prolate-spheroidal shape, one with some free yolk and one with a small yolk, were laid after the frequency constants and correlation coefficients had been calculated. These eggs are included in Table I, but not in Table II, III, IV, or V.

TABLE II.—Frequency distribution of the variation in size and shape of the several classes of dwarf eggs—Continued.

Class.	EGG WEIGHT			Frequency of cylindrical shape.	
	Frequency of prolate-spheroidal shape.			Yolkless.	Some free yolk.
	Yolkless.	Some free yolks.	A small yolk.		
<i>Gm.</i>					
3. 0-5. 9.....	2	1	.....	1	1
6. 0-8. 9.....	6	7	.....	0	0
9. 0-11. 9.....	10	17	.....	2	1
12. 0-14. 9.....	9	34	1	1	2
15. 0-17. 9.....	21	18	5	0	.....
18. 0-20. 9.....	15	12	2	3	.....
21. 0-23. 9.....	9	15	3	0	.....
24. 0-26. 9.....	5	16	3	1	.....
27. 0-29. 9.....	6	9	6	.....	.....
30. 0-32. 9.....	.....	3	4	.....	.....
33. 0-35. 9.....	.....	5	2	.....	.....
36. 0-38. 9.....	.....	1	.....	.....	.....
Total.....	83	<sup>a</sup> 138	<sup>a</sup> 26	8	4

INDEX

<i>Per cent.</i>					
34. 0-36. 9.....	.....	.....	.....	.....	1
37. 0-39. 9.....	.....	.....	.....	.....	0
40. 0-42. 9.....	.....	.....	.....	.....	0
43. 0-45. 9.....	.....	.....	.....	.....	0
46. 0-48. 9.....	.....	.....	.....	1	1
49. 0-51. 9.....	.....	.....	.....	0	1
52. 0-54. 9.....	.....	.....	.....	1	0
55. 0-57. 9.....	.....	.....	.....	3	0
58. 0-60. 9.....	.....	.....	.....	1	1
61. 0-63. 9.....	.....	.....	.....	1	.....
64. 0-66. 9.....	1	3	1	1	.....
67. 0-69. 9.....	2	5	1	.....	.....
70. 0-72. 9.....	3	6	0	.....	.....
73. 0-75. 9.....	16	14	2	.....	.....
76. 0-78. 9.....	11	15	9	.....	.....
79. 0-81. 9.....	12	21	6	.....	.....
82. 0-84. 9.....	17	31	6	.....	.....
85. 0-87. 9.....	12	25	1	.....	.....
88. 0-90. 9.....	6	10	.....	.....	.....
91. 0-93. 9.....	2	7	.....	.....	.....
94. 0-96. 9.....	1	1	.....	.....	.....
Total.....	83	<sup>a</sup> 138	<sup>a</sup> 26	8	4

<sup>a</sup> Two dwarf eggs of prolate-spheroidal shape, one with some free yolk and one with a small yolk, were laid after the frequency constants and correlation coefficients had been calculated. These eggs are included in Table I, but not in Table II, III, IV, or V.

The size and variation of the different egg parts in dwarf eggs is an interesting but difficult question. It was found possible to separate accurately the parts in a dwarf egg with a small yolk inclosed in yolk

membrane. The method employed was the one in routine use at this laboratory (3). The egg was first weighed; then the egg was broken and the parts separated. The yolk and shell were wiped as dry as possible with filter paper and weighed. The weight of albumen was determined by difference. The weights of the parts were determined for 16 of the small-yolked dwarf eggs. This number is so small that the variation and correlation were studied directly from the ungrouped data. These data are given in Table III.

The frequency constants calculated from the distributions in Table II and from the data in Table III are given in Table IV.

TABLE III.—*Weight of egg and of each of the egg parts for the 16 dwarf eggs on which these data were taken*

Egg No.	Weight of egg.	Weight of yolk.	Weight of albumen.	Weight of shell.
	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
1.....	20.00	0.85	15.50	3.65
2.....	17.00	1.00	12.00	4.00
3.....	18.40	1.85	12.75	3.80
4.....	24.50	2.19	19.09	3.22
5.....	20.00	2.50	12.50	5.00
6.....	22.50	3.55	16.70	2.25
7.....	24.85	4.37	17.10	3.38
8.....	29.00	4.50	20.00	4.50
9.....	24.75	4.53	16.17	4.05
10.....	30.00	5.00	21.25	3.75
11.....	29.00	5.50	20.00	3.50
12.....	31.00	5.50	21.00	4.50
13.....	32.50	6.50	18.50	7.50
14.....	30.33	6.50	19.83	4.00
15.....	32.00	7.00	20.25	4.75
16.....	34.00	7.00	22.00	5.00
Mean.....	26.24 ± 0.82	4.27 ± 0.34	17.79 ± 0.53	4.18 ± 0.19

The variation constants were not calculated for cylindrical eggs either with or without yolk, since the number is so small that these constants would be meaningless. The arithmetical mean in these cases was calculated directly from the data. For the sake of comparison the Table IV also gives the constants determined by Curtis (4) from the 3,180 normal eggs laid by a flock of 22 Barred Plymouth Rock birds during their pullet year, and the constants determined by Pearl and Surface (20) for the 450 eggs laid by an 850-bird flock of Barred Plymouth Rock pullets on February 12, 1908. The constants from the two series agree closely and may be considered a fair measure of the variation in the physical characters of the normal Barred Plymouth Rock egg. Since the second set of constants is based on a group of eggs, no two of which were laid by the same bird, they are theoretically the better measure of a random sample of Barred Plymouth Rock eggs.

TABLE IV.—Constants of variation in size and shape in the several types of dwarf eggs and in normal eggs

Character.	Dwarf eggs.					All normal first-year eggs from 22-bird flock.	All eggs laid by 850 birds on Feb. 12, 1908.
	Prolate-spheroidal shape.			Cylindrical shape.			
	Yolkless.	Some free yolk.	A small yolk.	Yolkless.	Some free yolk.		
Number of eggs.....	83	138	26	8	4	3,180	450
Length:							
Mean.....	35.27±0.37	35.84±0.34	41.35±0.50	41.68	31.3	55.70±0.03	56.32±0.08
Standard deviation.....	5.03±.26	5.88±.24	3.81±.36			2.41±.02	2.39±.05
Coefficient of variation.....	14.26±.76	16.40±.68	9.22±.87			4.33±.04	4.24±.09
Breadth:							
Mean.....	28.23±.24	28.94±.21	32.38±.38	23.59	15.73	41.14±.02	41.92±.04
Standard deviation.....	3.20±.17	3.61±.15	2.87±.27			1.41±.01	1.38±.03
Coefficient of variation.....	11.34±.60	12.48±.51	8.85±.83			3.44±.03	3.29±.07
Index:							
Mean.....	80.75±.44	81.54±.36	78.88±.59	57.57	48.67	73.95±.04	74.52±.12
Standard deviation.....	5.96±.31	6.31±.26	4.41±.42			3.30±.03	3.79±.09
Weight:							
Mean.....	17.11±.43	18.35±.41	24.81±.81	15.24	10.63	52.92±.06	55.26±.15
Standard deviation.....	5.86±.31	7.14±.29	6.13±.57			5.01±.04	4.62±.10
Coefficient of variation.....	34.24±1.99	38.90±1.80	24.70±2.45			9.46±.08	8.36±.19
Weight:							
Mean.....			26.24±.82				
Standard deviation.....			5.29±.63				
Coefficient of variation.....			20.15±2.50				
Yolk weight:							
Mean.....			4.27±.34			15.77±.02	
Standard deviation.....			2.01±.24			1.78±.02	
Coefficient of variation.....			47.12±6.75			11.31±.11	
Albumen weight:							
Mean.....			17.79±.53			31.55±.05	
Standard deviation.....			3.15±.37			3.87±.04	
Coefficient of variation.....			17.69±2.18			12.27±.11	
Shell weight:							
Mean.....			4.18±.19			5.12±.01	
Standard deviation.....			1.10±.13			.71±.01	
Coefficient of variation.....			26.31±3.35			13.86±.13	

<sup>a</sup> Calculated directly from the data (Table III) for the 16 eggs on which the weights of the parts were known.

The flocks which produced the dwarf eggs were largely composed of Barred Plymouth Rock birds from 5 to 17 months of age—that is, the birds were for the most part of the same age and strain as those producing the eggs from which the normal variation constants were calculated. Most of the dwarf eggs were produced by birds of this age and strain. A few were produced by birds of other breeds and a few by older birds. Barred Plymouth Rock birds in their first year produced dwarf eggs which extend over the whole range of size and shape. The slight heterogeneity of the material can not have so materially affected



the variation constants that it is unfair to compare them with the constants for normal Barred Plymouth Rock eggs.

By means of the data given in Tables II, III, and IV it is possible to compare the size, shape, and degree of variability of the several groups of dwarf eggs both among themselves and with normal eggs.

A.—SIZE RELATION OF DWARF AND NORMAL EGGS

The means given in Table IV show mathematically that all classes of dwarf eggs are of lighter weight and both shorter and narrower than normal eggs. This fact is, of course, obvious from the most casual inspection of dwarf and normal eggs. In comparing the different classes of dwarf eggs with each other it is necessary to keep in mind that the number of cylindrical eggs is so small that the means determined may not represent the true means for this class of eggs. Of the eggs studied, however, the mean prolate-spheroidal, or egg-shaped, egg was decidedly heavier than the mean cylindrical egg. It was also decidedly broader. It can be seen from the means given in the table that the mean weight and the mean breadth for both groups of cylindrical eggs are smaller than the mean for the same character for any group of the prolate-spheroidal eggs. The mean lengths for all cylindrical and all prolate-spheroidal eggs may be compared by calculating from the means in Table IV the weighted mean for each of these shape groups. The mean length for the cylindrical eggs is 38.22 and for the prolate-spheroidal eggs it is 36.23—that is, the cylindrical eggs studied were much lighter in weight, decidedly narrower, but slightly longer than the eggs of the prolate-spheroidal type.

The number of each class of cylindrical eggs is so small that the comparisons of the means for the two classes is of very doubtful meaning. A comparison of the means for the several groups of prolate-spheroidal eggs seems to show that those with small yolks average longer, broader, and heavier than those of the other groups, while the means for the dwarf eggs with some yolk not in membrane (free yolk) are slightly higher than for yolkless dwarf eggs. While the number of dwarf eggs in each group of prolate-spheroidal eggs is larger than in the case of cylindrical dwarf eggs, the actual number is not very large. In order to determine whether or not the above noted differences are greater than those which might arise from errors in sampling, each difference is compared with its probable error. The first section of Table V gives for each physical character measured the deviation in mean with the probable error, and the ratio of the error to the deviation between normal eggs<sup>1</sup>

<sup>1</sup> Since the constants derived from the 450 eggs laid on the same day are measures of an absolutely random sample of Barred Plymouth Rock eggs, these constants are used in calculating the difference between dwarf and normal eggs in the case of length, breadth, index, and weight. Data on the weight of the egg parts were not taken on the 450 egg series. Therefore the only available constants for these characters are those determined from all of the first-year eggs of the small flock.

and small-yolked dwarf eggs, between small-yolked and free-yolked dwarf eggs, and between free-yolked and yolkless dwarf eggs.

TABLE V.—Deviation between normal eggs and egg-shaped dwarf eggs and between the different classes of egg-shaped dwarf eggs for the mean and coefficient of variation of each measured character

Character.	Classes compared.	Difference in mean with probable error.	Difference $\pm$ Probable error of a difference.
Length . . . . .	Normal <i>a</i> —small-yolked dwarf . . . . .	14.97 $\pm$ 0.51	29.4
Do . . . . .	Small-yolked—free-yolked dwarf . . . . .	5.51 $\pm$ .60	9.2
Do . . . . .	Free-yolked—yolkless dwarf . . . . .	.57 $\pm$ .50	1.1
Breadth . . . . .	Normal <i>a</i> —small-yolked dwarf . . . . .	9.54 $\pm$ .38	24.5
Do . . . . .	Small-yolked—free-yolked dwarf . . . . .	3.14 $\pm$ .43	8.0
Do . . . . .	Free-yolked—yolkless dwarf . . . . .	.71 $\pm$ .32	2.2
Index . . . . .	Normal <i>a</i> —small-yolked dwarf . . . . .	-4.36 $\pm$ .60	7.3
Do . . . . .	Small-yolked—free-yolked dwarf . . . . .	-2.66 $\pm$ .69	3.9
Do . . . . .	Free-yolked—yolkless dwarf . . . . .	.79 $\pm$ .57	1.4
Weight . . . . .	Normal <i>a</i> —small-yolked dwarf . . . . .	30.45 $\pm$ .82	37.1
Do . . . . .	Small-yolked—free-yolked dwarf . . . . .	6.46 $\pm$ .91	7.1
Do . . . . .	Free-yolked—yolkless dwarf . . . . .	1.24 $\pm$ .59	2.1
Yolk weight . . . . .	Normal <i>b</i> —small-yolked dwarf <i>c</i> . . . . .	11.50 $\pm$ .34	33.8
Albumen weight . . . . .	. . . . .do . . . . .	13.76 $\pm$ .53	26.0
Shell weight . . . . .	. . . . .do . . . . .	.94 $\pm$ .19	4.9

Character.	Classes compared.	Deviation in coefficient of variation with probable error of difference.	Deviation in coefficient of variation $\pm$ Probable error of difference.
Length . . . . .	Free-yolked—yolkless dwarf . . . . .	2.14 $\pm$ 1.02	2.1
Do . . . . .	Yolkless—small-yolked dwarf . . . . .	5.04 $\pm$ 1.15	4.4
Do . . . . .	Small-yolked dwarf—normal <i>a</i> . . . . .	4.98 $\pm$ .87	5.7
Breadth . . . . .	Free-yolked—yolkless dwarf . . . . .	1.14 $\pm$ .79	1.4
Do . . . . .	Yolkless—small-yolked dwarf . . . . .	2.49 $\pm$ 1.02	2.4
Do . . . . .	Small-yolked dwarf—normal <i>a</i> . . . . .	5.56 $\pm$ .83	6.7
Weight . . . . .	Free-yolked—yolkless dwarf . . . . .	4.66 $\pm$ 2.68	1.7
Do . . . . .	Yolkless—small-yolked dwarf . . . . .	9.54 $\pm$ 3.16	3.0
Do . . . . .	Small-yolked dwarf—normal <i>a</i> . . . . .	16.34 $\pm$ 2.46	6.6
Yolk weight . . . . .	Small-yolked dwarf <i>c</i> —normal <i>b</i> . . . . .	35.81 $\pm$ 6.75	5.3
Albumen weight . . . . .	. . . . .do . . . . .	5.42 $\pm$ 2.18	2.5
Shell weight . . . . .	. . . . .do . . . . .	12.45 $\pm$ 3.35	3.7

*a* Calculated from the 450 eggs laid by the flock on a single day.

*b* Calculated from all of the 3,180 eggs laid by a flock of 22 pullets during their first laying year.

*c* Calculated direct from the data for the 16 small-yolked dwarf eggs for which the weights of the parts were known.

It is customary to consider a difference smaller than twice the probable error as probably not significant, a difference between two and three times its probable error as of a doubtful significance, and a difference three or more times the error as certainly or almost certainly significant.

Pearl and Miner (17) have published a table showing, for each value of the ratio of the probable error to the deviation, the probable occurrence in a hundred trials of a deviation as great or greater than the observed, provided chance alone is operating, and also the odds against its occurrence. From this table we see that the odds against a deviation due to chance alone, which is 3.0 times its probable error, is 22.26 to 1. We also see that above 3.0 the increase in odds is very rapid. At 4.0 it is 142.26 to 1; at 5.0 it is 1,350.35 to 1; at 8.0 it is 1,470,588,234 to 1. In the present discussion a deviation less than twice its probable error is considered insignificant. The significance of a deviation between two and three times the probable error is considered doubtful. A deviation between three and four times its probable error is considered probably significant. A deviation four or more times its probable error is considered almost certainly significant, with the understanding that when the odds against the occurrence of a given deviation being due to chance alone are as great or greater than 142.26 to 1, the deviation is almost certainly due to some other cause than error of sampling.

From Table V we see that small-yolked dwarf eggs are significantly smaller than normal eggs and larger than the other classes of dwarf eggs. These significant differences are seen in length, breadth, and weight<sup>1</sup>—that is, the small-yolked egg is nearer the size of a normal egg than are dwarf eggs with little or no yolk. The average length, breadth, and weight are all slightly higher for dwarf eggs which contain some free yolk than for yolkless dwarf eggs. These slight differences may be due to errors in sampling, since in no case is the deviation three times its probable error—that is, although the mean size of the observed dwarf eggs with some free yolk is slightly greater than the mean size of the observed yolkless dwarf eggs, this difference is not certainly significant.

These results are in line with the results from other investigations on the size of eggs. First, Pearl (12) showed that the relation of the weight of the entire egg to the number of yolks contained (zero, one, two, or three) is very accurately described by a parabola. He concluded that, while the size of eggs is not directly proportional to the number of yolks they contain, a definite relation probably exists between the amount of albumen secreted and the amount of yolk present in the duct in a given case. Second, Curtis (5) showed that within the eggs of an individual bird the actual weight of both albumen and shell is higher in triple-yolked than in double-yolked and higher in double-yolked than in single-yolked eggs. The increase in these accessory parts is not, however, proportional to the increase in yolk weight, since the yolk which formed only 24.37 per cent of the normal eggs formed 33.91 per cent of the double-yolked and 35.52 per cent of the triple-yolked eggs. Third, Curtis (4) showed that in the normal eggs of each individual bird there is a significant

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<sup>1</sup> The weight of each egg part is also significantly smaller in small-yolked dwarf than in normal eggs.

correlation between the weight of the yolk and the weight of the albumen—that is, the amount of albumen secreted is in part at least dependent on the amount of immediate stimulation due to the quantity of yolk in the duct.

The results recorded for the different classes of dwarf eggs carry these results further. The eggs which contain small-formed yolks are smaller than normal eggs and larger than eggs which contain either little or no yolk. That eggs with a small amount of free yolk are not certainly significantly larger than eggs without yolk is explained by the fact that the two groups were separated strictly on a basis of the presence or absence of yolk. Dwarf eggs which do not contain formed yolks contain as nuclei lumps or drops of free yolk, lumps of hardened secretion, blood clots, or fibers of coagulated albumen. The size of these nuclei vary considerably. A single drop or a very small lump of yolk threw the egg into the class of free-yolked dwarf eggs. Several yolkless dwarf eggs contained nuclei larger than some of the lumps or drops of yolk found in the free-yolked dwarf eggs. In a broad way at least the size of the egg varies with the size of the nucleus—that is, a large dwarf egg contains a considerable amount of yolk or some other large nucleus. A very small one contains a small nucleus. Since the irregular particles can not be accurately measured, the degree of this relationship<sup>1</sup> can not be ascertained.

A comparison of the mean egg size of the several groups of dwarf eggs classified according to yolk content confirms the evidence obtained from a study of normal and multiple-yolked eggs that the amount of yolk (or other nucleus) present in the oviduct is an important factor in determining the amount of albumen secreted in a given case.

#### B.—RELATIVE SHAPE OF DWARF AND NORMAL EGGS

Tables IV and V also give data for a study of the comparative shape of the several classes of dwarf and of normal eggs. It has already been noted that there are two distinct shape groups of dwarf eggs: Cylindrical and prolate-spheroidal eggs. A comparison of the mean indices shows that cylindrical dwarf eggs are longer in proportion to their breadth than are normal eggs, while prolate-spheroidal eggs are proportionately shorter than normal eggs. It is also seen that dwarf eggs with small yolks are nearer the shape of normal eggs than are dwarf eggs without formed yolks.

The cause for the distinctly different form in cylindrical and prolate-spheroidal dwarf eggs can not be certainly decided from the material at hand. In several cases of cylindrical dwarf eggs the form of the nucleus was not noted. However, in a few pronounced cases it was noted that

<sup>1</sup> On page 1000 it is shown that in dwarf eggs with formed yolks the yolk weight is highly correlated both with the egg weight and the albumen weight.

the nucleus of coagulated fibers of albumen was drawn out in a line parallel to the long axis of the egg. Further, at one of our routine autopsies there was found in an oviduct a string of albumen 5 or 6 cm. long and not more than 1 cm. in diameter. This was wrapped around a long thread of coagulated albumen fibers which lay parallel to the length of the duct. It seems probable that the form of the stimulating nucleus is one of the factors in determining the shape of the egg. When the stimulus is small in amount and drawn out, the degree of stimulation must be small but the area covered large.

In the prolate-spheroidal eggs the nucleus is usually of globular form—that is, its shape is comparable to the shape of a normal yolk. All the eggs with small-formed yolks were of the prolate-spheroidal type. It has been noted that indices for dwarf eggs with small yolks are higher than those for normal eggs and lower than those for other prolate-spheroidal eggs. The order for the value of index is thus the reverse of the order for the size characters. Later it will be shown that within each group of dwarf eggs the index is negatively correlated with weight. In earlier investigations (3, 5) it has been shown, first, that the indices for multiple-yolked eggs lie below the range of variation for the indices of normal eggs, and, second, that within the normal eggs of an individual the index is negatively correlated with weight. The results from the study of dwarf eggs, therefore, extend the former evidence that the smaller the egg the broader it is in proportion to its length. Two factors may be working together to produce this negative correlation between index and weight. First, the greater the long diameter of the nucleus—be it yolk drop, normal yolk, or two or three yolks in tandem—the longer will be the area of oviduct stimulated at the same time; and, second, when a plastic body is forced (by peristalsis) through an elastic tube the tube will offer less mechanical resistance to the passage of a small than a large body. This mechanical factor is probably of great importance in determining the shape of the egg.

#### C.—RELATIVE VARIABILITY OF DWARF AND NORMAL EGGS

Tables IV and V give also the data for comparing the variability of the different classes of prolate-spheroidal dwarf eggs with each other and with normal eggs. Table IV gives for normal eggs and for each class of egg-shaped dwarf eggs the standard deviation for length, breadth, index, and weight, and the coefficient of variation for each of these characters except index.<sup>1</sup> In the case of normal eggs and dwarf eggs with formed yolks it also gives the variation constants for each egg part (yolk, albumen, and shell). A comparison either of standard deviations or of coefficients of variation shows that normal eggs are less variable in each character measured than are the eggs of any class of prolate-spheroidal dwarf eggs. The

<sup>1</sup> Coefficients of variation of percentage characters have no physical significance.

most variable class of dwarf eggs is apparently those which have some free yolk, while the yolkless dwarf eggs are more variable than the small-yolked dwarf eggs—that is, the small-yolked dwarf egg approaches the normal in degree of variability as well as in size and shape. In comparing classes where the absolute difference in size is as great as it is between normal and dwarf eggs the coefficients of variation are more accurate measures of relative variability than are the standard deviations. In order to determine whether or not the apparent difference in degree of variability shown by the several classes is significant, it is necessary to compare these differences with their probable errors. The second section of Table V shows these differences in the size characters with their probable errors and the ratio of each difference to its probable error. From this table we see, first, that normal eggs are significantly less variable than the least variable class of dwarf eggs (small-yolked dwarf eggs) in length, breadth, egg weight, yolk weight, and probably shell weight. The significance of the smaller variation in albumen weight is doubtful. Second, small-yolked dwarf eggs are almost certainly less variable than other dwarf eggs in length and probably also in weight. The significance of the smaller variation in breadth is doubtful. Third, the somewhat greater variation in every size character in the dwarf eggs with free yolk than in the yolkless eggs is not certainly significant—that is, it may be due to errors in sampling.

As previously stated, the coefficient of variation of index, which is a percentage character, has no physical meaning. Since the index equals the percentage that the breadth is of the length, all the indices are measured in the same units and have the same possibilities of variation in range. There is, then, less objection to comparing the standard deviations of such a character; in fact, such a comparison is the only available measure of the relative variability in shape of the several groups. However, too much reliance should not be placed on the figures. The differences in the standard deviations of the indices for the different groups are as follows:

Free-yolked—yolkless dwarf.....	=0.35±0.40
Yolkless—small yolked dwarf.....	=1.55±.52
Small-yolked dwarf—normal.....	=.62±.43

The only deviation which can be considered of even probable significance is the difference between yolkless and small-yolked dwarf eggs—that is, normal eggs and small-yolked dwarf eggs are probably less variable in shape than dwarf eggs without a formed yolk.

The relative variability of the size characters within each group is also of some interest. From Table IV it may be seen that the order of variability of the size characters of the egg is the same in normal eggs and in each class of the dwarf eggs. The size characters arranged in the order of their variability from most to least variable are (1) egg weight,

(2) length, and (3) breadth. In order to determine whether or not these apparent differences may be considered significant, the differences with their probable errors and the ratio of each difference to its probable error are given in Table VI.

TABLE VI.—Difference between the coefficients of variation in the size characters, together with the probable error of difference and the ratio of each difference to its probable error, for normal eggs and for each class of egg-shaped dwarf eggs

Class.	Characters compared.	Difference in coefficient of variation, with probable error.	Difference + probable error of difference.
Normal eggs <sup>a</sup> . . . . .	Egg weight—length . . . . .	4. 12 ± 0. 21	10. 6
Do. . . . .	Length—breadth . . . . .	. 95 ± . 11	8. 6
Small-yolked dwarf eggs. . . . .	Egg weight—length . . . . .	15. 48 ± 2. 60	6. 0
Do. . . . .	Length—breadth . . . . .	. 37 ± 1. 20	. 3
Free-yolked dwarf eggs . . . . .	Egg weight—length . . . . .	22. 50 ± 1. 92	11. 7
Do. . . . .	Length—breadth . . . . .	3. 92 ± . 85	4. 6
Yolkless dwarf eggs . . . . .	Egg weight—length . . . . .	19. 98 ± 2. 13	9. 4
Do. . . . .	Length—breadth . . . . .	2. 92 ± . 97	3. 0

<sup>a</sup> From 450 eggs laid by a flock of Barred Plymouth Rock pullets in a single day.

From Table VI it appears that the order of variability of the characters is probably significant—that is, in both normal and dwarf eggs the size characters may be arranged in the order of their variability as egg weight, length, and breadth. The only case where the difference is less than three times its probable error is between length and breath in “cock eggs” with small yolks. In this case the deviation might have been due to errors of sampling.

In normal eggs it has been shown by Curtis (4) that the weight of the whole egg is less variable than the weight of any part (yolk, albumen, or shell). Of the three parts the shell is the most and the yolk the least variable. The coefficients calculated for 16 dwarf eggs with small yolks do not show the same relative variability of the parts. In these eggs the weight of albumen was less variable than the weight of the whole egg, and the yolk weight instead of being the most constant of the three parts was the most variable. The number of eggs, however, is so small that the probable error of sampling is large. Table VII shows that when the coefficients of variation of the egg parts are arranged in the order of their value, the differences between the two of nearest value is in no case equal to three times the probable error of difference. It is, however, probable that yolk weight is more variable compared to the weight of the other parts and to the whole egg in small-yolked dwarf than in normal eggs.

TABLE VII.—Relative variability of the whole and the several parts of normal eggs and of dwarf eggs with small yolks

NORMAL EGGS <sup>a</sup>		
Characters compared.	Difference in coefficient of variation with probable error.	Difference $\pm$ probable error of difference.
Shell weight—albumen weight.....	1. 59 $\pm$ 0. 17	9. 4
Albumen weight—yolk weight.....	. 90 $\pm$ . 16	6. 0
Yolk weight—egg weight.....	1. 85 $\pm$ . 14	13. 2

SMALL-YOLKED DWARF EGGS <sup>b</sup>		
Yolk weight—shell weight.....	20. 81 $\pm$ 7. 54	2. 8
Shell weight—albumen weight.....	8. 62 $\pm$ 4. 00	2. 1
Egg weight—albumen weight.....	2. 46 $\pm$ 3. 32	. 7

<sup>a</sup> From 3,180 eggs laid by flock of 22 Barred Plymouth Rock pullets.<sup>b</sup> Coefficients calculated direct from data for the 16 dwarf eggs of known yolk weight.

#### IV.—INTERRELATION OF THE DIMENSIONS, SHAPE, AND WEIGHT OF EACH CLASS OF DWARF EGGS COMPARED TO THE SAME RELATIONS IN NORMAL EGGS

We have seen that the dwarf eggs of each group vary greatly in each dimension and in weight and shape. We shall now consider the correlation in the variation of the several characters in prolate-spheroidal<sup>1</sup> dwarf eggs of each class. It will be determined whether a long dwarf egg is broader or narrower than a short one of the same class; whether a large dwarf egg of any class is longer or broader or both longer and broader than a small egg of the same class; whether a large dwarf egg is longer or shorter in proportion to its breadth than a small dwarf of the same group; and whether or not these relations are the same in the several groups of dwarf eggs and in normal eggs. In the case of dwarf eggs with formed yolk the relation between yolk weight and albumen weight is also studied.

The correlations studied then are length with breadth, breadth with weight, length with weight, index with weight, yolk weight with egg weight, and yolk weight with albumen weight. On account of the small number of dwarf eggs of known yolk weight, the correlations involving yolk weight were calculated directly from the data given in Table III. In the case of the other pairs of characters the usual correlation tables were made for each class of dwarf eggs. These are shown as Tables VIII to XIX, inclusive.

<sup>1</sup> Cylindrical eggs appear to show the same relations among themselves as the prolate-spheroidal eggs, but the number is too small to determine the significance of the relationship.



TABLE VIII.—Correlation between egg breadth and egg length in dwarf eggs with some yolk not in a yolk membrane

Egg length (in millimeters).	Egg breadth (in millimeters).									Total.	
	18.00-19.99	20.00-21.99	22.00-23.99	24.00-25.99	26.00-27.99	28.00-29.99	30.00-31.99	32.00-33.99	34.00-35.99		36.00-37.99
20.00-22.99	1										1
23.00-25.99	1										1
26.00-28.99	1										1
29.00-31.99		1									1
32.00-34.99			3	5	2						11
35.00-37.99			1	5	7	22					32
38.00-40.99				1	5	9	10				25
41.00-43.99					1	2					16
44.00-46.99							8	5			13
47.00-49.99							1	7	7	1	23
50.00-52.99								4	5	5	16
Total	1	3	5	18	35	25	20	18	9	4	138

TABLE IX.—Correlation between egg breadth and egg length in dwarf eggs without yolk

Egg length (in millimeters).	Egg breadth (in millimeters).									Total.	
	18.00-19.99	20.00-21.99	22.00-23.99	24.00-25.99	26.00-27.99	28.00-29.99	30.00-31.99	32.00-33.99	34.00-35.99		
20.00-22.99	1										1
23.00-25.99		1	1								2
26.00-28.99			1	3	1						5
29.00-31.99			1	3	3	6	1				14
32.00-34.99					3	2	10	1			16
35.00-37.99						6	8	9			23
38.00-40.99						1	3	4	2	1	11
41.00-43.99							1	4	1		6
44.00-46.99								2	3		5
Total	1	3	7	7	15	23	20	6	1		83

TABLE X.—Correlation between egg breadth and egg length in dwarf eggs with small yolks

Egg length (in millimeters).	Egg breadth (in millimeters).							Total.
	26.00-27.99	28.00-29.99	30.00-31.99	32.00-33.99	34.00-35.99	36.00-37.99	38.00-39.99	
32.00-34.99		1						1
35.00-37.99		4	2					6
38.00-40.99			2	1	1			4
41.00-43.99	1	1	1	3		1		7
44.00-46.99				1	6			7
47.00-49.99							1	1
Total	1	6	5	5	7	1	1	26

TABLE XI.—Correlation between egg weight and egg length in dwarf eggs with some yolk not in a yolk membrane

Egg length (in millimeters).	Egg weight (in grams).										Total.		
	3.00-5.99	6.00-8.99	9.00-11.99	12.00-14.99	15.00-17.99	18.00-20.99	21.00-23.99	24.00-26.99	27.00-29.99	30.00-32.99		33.00-35.99	36.00-38.99
20.00-22.99.....	I												I
23.00-25.99.....		I											I
26.00-28.99.....		5	5										II
29.00-31.99.....		I	IO	20	I								32
32.00-34.99.....			2	IO	I2	I							25
35.00-37.99.....				3	4	5	4						16
38.00-40.99.....					I	5	6	8					23
41.00-43.99.....						I	2	7	4	I	I		16
44.00-46.99.....								I		2		4	7
47.00-49.99.....							I		2	I		I	5
50.00-52.99.....										I			I
Total.....	I	7	17	34	18	12	15	16	9	3	5	I	138

TABLE XII.—Correlation between egg weight and egg length in dwarf eggs without yolk

Egg length (in millimeters).	Egg weight (in grams).								Total.	
	3.00-5.99	6.00-8.99	9.00-11.99	12.00-14.99	15.00-17.99	18.00-20.99	21.00-23.99	24.00-26.99		27.00-29.99
20.00-22.99.....	I									I
23.00-25.99.....	I	I								2
26.00-28.99.....		3	2							5
29.00-31.99.....		I	7	4	I	I				14
32.00-34.99.....		I	I	3	II					16
35.00-37.99.....				2	8	IO	3			23
38.00-40.99.....					I	4	3	2	I	11
41.00-43.99.....							3	2	I	6
44.00-46.99.....								I	4	5
Total.....	2	6	10	9	21	15	9	5	6	83

TABLE XIII.—Correlation between egg weight and egg length in dwarf eggs with small yolks

Egg length (in millimeters).	Egg weight (in grams).								Total.
	12.00-14.99	15.00-17.99	18.00-20.99	21.00-23.99	24.00-26.99	27.00-29.99	30.00-32.99	33.00-35.99	
32.00-34.99.....		I							I
35.00-37.99.....		I	3	2					6
38.00-40.99.....				2	I	I			4
41.00-43.99.....		I		I	2	2		I	7
44.00-46.99.....						2	4	I	7
47.00-49.99.....						I			I
Total.....	I	5	2	3	3	6	4	2	26

TABLE XIV.—*Correlation between egg weight and egg breadth in dwarf eggs with some yolk and not in a yolk membrane*

Egg breadth (in millimeters).	Egg weight (in grams).										Total.		
	3.00-5.99	6.00-8.99	9.00-11.99	12.00-14.99	15.00-17.99	18.00-20.99	21.00-23.99	24.00-26.99	27.00-29.99	30.00-32.99		33.00-35.99	36.00-38.99
18.00-19.99.....	I												I
20.00-21.99.....		3											3
22.00-23.99.....		3	I		I								5
24.00-25.99.....		I	II	5	I								18
26.00-27.99.....			5	27	3								35
28.00-29.99.....				2	13	8	2						25
30.00-31.99.....						4	II	4	I				20
32.00-33.99.....							2	9	5	2			18
34.00-35.99.....							2	3	I	3			9
36.00-37.99.....								I		2	I		4
Total.....	I	7	17	34	18	12	15	16	9	3	5	I	138

TABLE XV.—*Correlation between egg weight and egg breadth in dwarf eggs without yolk*

Egg breadth (in millimeters).	Egg weight (in grams).								Total.	
	3.00-5.99	6.00-8.99	9.00-11.99	12.00-14.99	15.00-17.99	18.00-20.99	21.00-23.99	24.00-26.99		27.00-29.99
18.00-19.99.....	I									I
20.00-21.99.....	I	2								3
22.00-23.99.....		3	3							7
24.00-25.99.....		I	5	I			I			7
26.00-27.99.....			2	7	6					15
28.00-29.99.....			2	I	14	7	I			23
30.00-31.99.....					I	7	8			20
32.00-33.99.....								3	3	6
34.00-35.99.....									I	I
Total.....	2	6	10	9	21	15	9	5	6	83

TABLE XVI.—*Correlation between egg weight and egg breadth in dwarf eggs with small yolks*

Egg breadth (in millimeters).	Egg weight (in grams).								Total.
	12.00-14.99	15.00-17.99	18.00-20.99	21.00-23.99	24.00-26.99	27.00-29.99	30.00-32.99	33.00-35.99	
26.00-27.99.....		I							I
28.00-29.99.....		4	I	I					6
30.00-31.99.....	I		I	2	I				5
32.00-33.99.....					2	3			5
34.00-35.99.....						2	4		7
36.00-37.99.....								I	I
38.00-39.99.....						I			I
Total.....	I	5	2	3	3	6	4	2	26

TABLE XVII.—Correlation between egg weight and egg index in dwarf eggs with some yolk not in a yolk membrane

Egg index (in percentage).	Egg weight (in grams).										Total.		
	3.00-5.99	6.00-8.99	9.00-11.99	12.00-14.99	15.00-17.99	18.00-20.99	21.00-23.99	24.00-26.99	27.00-29.99	30.00-32.99		33.00-35.99	36.00-38.99
64.00-66.99									I	2			3
67.00-69.99				I		I	I	I	I				5
76.00-72.99		I			2	I	I		I				5
73.00-75.99			I	3	2	2	3	2	I				14
76.00-78.99			2	2	I	2	I	3	2		I	I	15
79.00-81.99		2		3	3	2	5	3	I	I	I		21
82.00-84.99		I	3	10	5	3	2	3	I		3		31
85.00-87.99			6	7	3		2	4	I				25
88.00-90.99		2	2	3	2	I							10
91.00-93.99	I	I	I	4									7
94.00-96.99				I									I
Total	I	7	17	34	18	12	15	16	9	3	5	I	138

TABLE XVIII.—Correlation between egg weight and egg index in dwarf eggs without yolk

Egg index (in percentage).	Egg weight (in grams).								Total.	
	3.00-5.99	6.00-8.99	9.00-11.99	12.00-14.99	15.00-17.99	18.00-20.99	21.00-23.99	24.00-26.99		27.00-29.99
64.00-66.99									I	I
67.00-69.99									2	2
70.00-72.99					I		I		I	3
73.00-75.99		I	I	2	2	4	2	3	I	16
76.00-78.99	I	I	I		4	I	2		I	11
79.00-81.99		I	I	2	3	4			I	12
82.00-84.99		I	2	2	4	6	I	I		17
85.00-87.99		I	I	2	4		3		I	12
88.00-90.99	I		3	I	I					6
91.00-93.99		I			I					2
94.00-96.99					I					I
Total	2	6	10	9	21	15	9	5	6	83

TABLE XIX.—Correlation between egg weight and egg index in dwarf eggs with small yolks

Egg index (in percentage).	Egg weight (in grams).								
	12.00-14.99	15.00-17.99	18.00-20.99	21.00-23.99	24.00-26.99	27.00-29.99	30.00-32.99	33.00-35.99	Total.
65.00-67.99.....		I		I					2
68.00-70.99.....									0
71.00-73.99.....						I			I
74.00-76.99.....					I		2		3
77.00-79.99.....			I	I	I	4	2	I	10
80.00-82.99.....		3	I	I					5
83.00-85.99.....	I	I			I	I		I	5
Total.....	I	5	2	3	3	6	4	2	26

Table XX shows the correlation coefficients deduced from Tables VIII to XIX (or in the case of yolk weight from the data) with their probable errors, and also similar coefficients for normal eggs. The correlations are all calculated from the usual Bravais formula

$$r = \frac{S(xy)}{N\sigma_1\sigma_2}$$

where  $N$  = number of eggs;  $x$  and  $y$  are the deviations from the means and  $\sigma_1$  and  $\sigma_2$  the standard deviations of the two variables.

TABLE XX.—Correlation coefficients for the size and shape characters of the dwarf and normal eggs

Kind of eggs.	Number of eggs.	Correlation coefficients.					
		Length and breadth.	Length and weight.	Breadth and weight.	Index and weight.	Yolk weight and egg weight.	Yolk weight and albumen weight.
Free-yolked dwarf.....	138	0.874 ± 0.013	0.933 ± 0.007	0.950 ± 0.006	-0.408 ± 0.048		
Yolkless dwarf....	83	.844 ± .021	.924 ± .011	.919 ± .011	-.368 ± .064		
Small-yolked dwarf.....	26	.759 ± .056	.840 ± .039	.883 ± .029	-.092 ± .131	0.940 ± 0.020	0.789 ± 0.064
Normal (mean of individual coefficients for 22 birds).....	3,180	.378	.753	.830	-.185	.818	.566
Normal (eggs laid by flock on a single day).....	450	.084 ± .032	.580 ± .021	.836 ± .010	-.085 ± .032		

<sup>a</sup> These coefficients were calculated directly from the data given in Table III.

Table XXI gives the differences between various of these correlation coefficients, the probable error of the differences, and the ratio of each difference to its probable error.

TABLE XXI.—*Deviation in correlation coefficients, the probable error of the differences, and the ratio of each difference to its probable error*

Class of eggs.	Pairs of characters, the correlation coefficients of which are compared.	Difference in correlation coefficients with probable error.	Difference + probable error of difference.
Free-yolked dwarf.....	Breadth with weight—length with weight.....	0.017 ± 0.009	2.0
Do.....	Length with weight—length with breadth.....	.059 ± .015	3.9
Do.....	Length with breadth—index with weight.....	.466 ± .050	9.3
Yolkless dwarf.....	Breadth with weight—length with weight.....	-.005 ± .016	.3
Do.....	Length with weight—length with breadth.....	.080 ± .024	3.3
Do.....	Length with breadth—index with weight.....	.476 ± .067	7.1
Small-yolked dwarf.....	Breadth with weight—length with weight.....	.043 ± .049	.9
Do.....	Length with weight—length with breadth.....	.081 ± .068	1.2
Do.....	Length with breadth—index with weight.....	.667 ± .142	4.7
Normal.....	Breadth with weight—length with weight.....	.256 ± .023	11.1
Do.....	Length with weight—length with breadth.....	.496 ± .038	13.1
Do.....	Length with breadth—index with weight.....	-.001 ± .045	.02

Pairs of characters correlated.	Classes compared.	Difference in correlation coefficients with probable error.	Difference + probable error of difference.
Length with breadth.....	Free-yolked—yolkless.....	0.030 ± 0.025	1.2
Do.....	Yolkless—small-yolked.....	.085 ± .060	1.4
Do.....	Small-yolked—normal.....	.675 ± .065	10.4
Length with weight.....	Free-yolked—yolkless.....	.009 ± .013	.7
Do.....	Yolkless—small-yolked.....	.084 ± .041	2.1
Do.....	Small-yolked—normal.....	.260 ± .044	5.9
Breadth with weight.....	Free-yolked—yolkless.....	.031 ± .013	2.4
Do.....	Yolkless—small-yolked.....	.036 ± .031	1.2
Do.....	Small-yolked—normal.....	.047 ± .031	1.5
Index with weight.....	Free-yolked—yolkless.....	.010 ± .080	2.5
Do.....	Yolkless—small-yolked.....	.276 ± .146	1.9
Do.....	Small-yolked—normal.....	.007 ± .135	.05
Yolk weight with egg weight.....	do.....	.122	.....
Yolk weight with albumen weight.....	do.....	.223	.....

From Table XX the following points may be noted:

1. In each class of dwarf eggs the correlation between the two dimensions is positive and is certainly significant—that is, a broad dwarf egg is also long, and vice versa. The shape of the egg is no doubt determined by the action of the longitudinal and circular muscle fibers of the oviduct walls, especially during the formation of the egg membrane and shell. The egg is a fluid body which tends to take a spherical shape when not under pressure. At the time an egg receives its membrane and shell a normal egg or almost any dwarf egg is larger than the normal diameter of the oviduct. It is therefore under pressure which tends to elongate it in the direction of the long axis of the duct. The degree of pressure and, hence, the resulting degree of elongation will depend on (a) the size of the egg compared to the diameter of a cross section of the duct, and

(b) the relative tonus of the two sets of muscle fibers of the oviduct wall. A decrease in the tonus of the circular fibers, or an increase in that of the longitudinal fibers, or both, may counterbalance the increase in pressure due to increase in the diameter of the egg. There is no a priori reason for assuming a correlation between breadth and length; in fact, this correlation was not significant in the random sample of normal eggs studied by Pearl and Surface (20). From this they concluded that the two sets of muscles are to a large extent independent in their action. On the other hand, Curtis (4) found that within the normal eggs of an individual there is usually <sup>1</sup> a significant correlation between length and breadth—that is, the size of the active oviduct and relative tonus of the two sets of muscle fibers in the oviduct wall are apparently usually relatively stable in an individual, and an increase in the breadth of the egg is correlated with an increase in the length. The fact that the correlation between length and breadth is significantly higher (Table XXI) for dwarf eggs than for normal eggs may indicate that in these small eggs there is little or no differential stimulus on the muscle fibers of the oviduct wall, but that there is such a stimulus when the egg is larger.

2. Length and breadth are both highly correlated with weight—that is, a heavy egg is both broad and long. These relations are also true for normal eggs. The random sample of eggs studied by Pearl and Surface (20) showed a correlation between breadth and weight which was significantly higher than the correlation between length and weight. The individual birds studied by Curtis (4) showed a great variation in the relative degree of correlation of the two dimensions with the weight. Half the flock showed a correlation for breadth and weight significantly higher than for length and weight. Two birds showed a higher length-weight correlation. For one-third of the flock the difference was insignificant. There is no significant difference between breadth-weight and length-weight correlation in any class of dwarf eggs. (See Table XXI.)

3. The index-weight correlations are negative, and they are significant for dwarf eggs with little or no yolk—that is, for those two groups of small dwarf eggs the larger the egg the longer it is in proportion to its breadth. In the study of the normal eggs of individual birds Curtis (4) found that there was a low negative correlation between index and weight which was significant for one-half of the individuals studied. This tendency toward a negative correlation between index and weight in dwarf and normal eggs is in line with the fact that the mean index of the several groups of dwarf eggs, normal eggs, and multiple-yolked eggs varies in the opposite direction from the mean egg weight of each group—that is, the larger the egg the lower the index. The bearing of this fact has already been discussed.

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<sup>1</sup> In 18 out of 22 individuals studied.

4. The correlation between yolk weight and egg weight in dwarf eggs with small yolks is very high. Since the yolk weight forms part of the egg weight, we will confine our discussion to the correlation between yolk weight and albumen weight. This correlation is also very high. It is in fact higher than the average correlation between yolk weight and albumen weight within the normal eggs of a single individual. This high correlation between yolk weight and albumen weight in dwarf eggs with small yolks adds to the evidence already presented that the amount of yolk present in the duct is an important factor in determining the amount of albumen secreted, and thus both directly and indirectly influences the size of the egg.

V.—FREQUENCY OF THE OCCURRENCE OF DWARF EGGS COMPARED TO NORMAL EGGS AND OF DWARF EGG PRODUCERS COMPARED TO BIRDS WHICH DO NOT LAY DWARF EGGS.

As previously stated, the period covered by this investigation extends from February 1, 1908, to February 1, 1916. During this period it has been the practice to make up the flock in September or early October. A few of the birds of the previous flock are saved for specific experiments and the rest killed or sold. The pullets are put in the houses at this time. The 298 dwarf eggs collected were thus produced by nine different flocks of birds. The number laid by each flock is given below:

	Number of dwarf eggs.
Feb. 1, 1908, to Aug. 31, 1908.....	16
Sept. 1, 1908, to Aug. 31, 1909.....	20
Sept. 1, 1909, to Aug. 31, 1910.....	34
Sept. 1, 1910, to Aug. 31, 1911.....	43
Sept. 1, 1911, to Aug. 31, 1912.....	59
Sept. 1, 1912, to Aug. 31, 1913.....	17
Sept. 1, 1913, to Aug. 31, 1914.....	27
Sept. 1, 1914, to Aug. 31, 1915.....	72
Sept. 1, 1915, to Feb. 1, 1916.....	10
Feb. 1, 1908, to Feb. 1, 1916.....	298

The first and last years are, of course, incomplete. The fluctuations between the other years are no doubt due largely to three causes. First, the size of the flock differs somewhat from year to year. Second, the average annual egg production fluctuates with changes in the proportion of low and high laying strains which compose the successive flocks—for example, the 1914-15 flock contained 55 less birds than the 1911-12 flock, and at the same time produced 25,374 more eggs, so that although it produced 22 per cent more dwarf eggs, the proportion of these eggs to the normal eggs was smaller. Third, as will be discussed later, certain birds suffer disturbances of physiology which cause them to produce a number of dwarf eggs. Such birds do not occur every year; in fact, an unusual proportion of the known cases occurred during the two years of highest dwarf-egg pro-



duction—that is, 1911-12 and 1914-15. During any year a few dwarf eggs may have escaped collection by being broken in the nest or laid on the floor and lost in the litter. This loss can not have been large at any time. However, in order to avoid the possibility of an unequal loss during the several years, the two years of highest dwarf-egg production were selected for a comparison as to the frequency of dwarf and normal eggs.

The frequency of the occurrence of dwarf eggs compared to normal eggs may be determined by calculating the percentage of all the eggs produced which are dwarf. For convenience this percentage may be multiplied by 100. This number represents the number of dwarfs in 10,000 eggs. This percentage was calculated for each of the 12 months of the two years taken both separately and combined. These data are given in Table XXII.

TABLE XXII.—Total egg production, dwarf-egg production, and number of dwarf eggs per 10,000 eggs for each month of the years 1911-12 and 1914-15 both separately and combined, also for the two years combined the percentage of all the eggs and of all of the dwarf eggs which were produced during each calendar month

Month.	1911-12			1914-15			1911-12 and 1914-15 combined.				
	Total eggs.	Dwarf eggs.	Dwarf eggs per 10,000 eggs.	Total eggs.	Dwarf eggs.	Dwarf eggs per 10,000 eggs.	Total eggs.	Dwarf eggs.	Dwarf eggs per 10,000 eggs.	Percentage of total number of eggs produced during month	Percentage of total number of dwarf eggs produced during month.
September.....	1,870	2	10.7	694	1	14.4	2,564	3	11.7	1.69	2.29
October.....	2,940	1	3.4	2,589	0	.0	5,529	1	1.8	3.64	.76
November.....	2,713	2	7.4	3,771	6	15.9	6,484	8	12.3	4.27	6.11
December.....	4,501	2	4.4	5,416	3	5.5	9,917	5	5.0	6.54	3.82
January.....	4,429	2	4.5	7,131	4	5.6	11,560	6	5.2	7.62	4.58
February.....	4,375	0	.0	8,409	3	3.5	12,784	3	2.3	8.43	2.29
March.....	9,407	7	7.4	9,728	5	5.1	19,135	12	6.3	12.61	9.16
April.....	7,886	7	8.9	11,406	8	7.0	19,292	15	7.8	12.71	11.45
May.....	7,738	10	12.9	11,051	7	6.3	18,789	17	9.0	12.38	12.98
June.....	7,121	17	23.9	10,080	10	9.9	17,201	27	15.7	11.34	20.61
July.....	5,539	2	3.6	9,618	20	20.7	15,157	22	14.5	9.99	16.79
August.....	4,657	7	15.0	8,667	5	5.7	13,324	12	9.0	8.78	9.16
Total....	63,176	59	9.3	88,560	72	8.1	151,736	131	8.6	100.0	100.0

The last line in Table XXII shows the total number of eggs, the total number of dwarf eggs, and the number of dwarf eggs per 10,000 for each of the two years, and for the two years combined. From these data it is seen that during the year 1911-12 the flock produced 59 dwarf eggs out of a total of 63,176, or 9.3 dwarf eggs in 10,000—that is, 1 dwarf egg in each 1,071 eggs. In 1914-15 the flock produced 72 dwarf eggs in a total of 88,560 eggs—that is, 8.1 dwarf eggs in 10,000, or 1 dwarf to 1,230 eggs. If the data for the two years are combined, there were produced 131 dwarf eggs in 151,736 eggs—that is, during the two years of maxi-

imum dwarf-egg production the proportion of dwarf to normal eggs was 8.6 dwarf eggs in 10,000, or 1 dwarf egg in 1,158 eggs. Warner and Kirkpatrick (26) show that during two laying contests at Storrs, Conn., 199,137 eggs were produced, of which 103 weighed less than 0.09 pound (40.82 gm.). From these figures we see that they obtained 5.2 dwarf eggs per 10,000, or 1 dwarf in 1,933 eggs.

The nine flocks which laid the dwarf eggs considered in this investigation contained approximately 4,800 different individual birds. Not all of these birds had an equal opportunity to lay a dwarf egg, for while a large majority of them were kept until, and only until, the end of their pullet year, a number died at varying ages and a number were kept for more than one year. Also the records for 1907-8 and 1915-16 are incomplete. We may, however, arrive at an approximate estimate of the proportion of birds which lay one or more eggs by neglecting these discrepancies and considering that each of the 4,800 individuals had an equal opportunity to produce dwarf eggs.

The 251 dwarf eggs of known origin were produced by 200 different individuals. There were 47 eggs laid by birds whose number was not known. Most of these were floor eggs. In a very few cases the poultryman neglected to record the number of the bird on the egg at collection time, and in a very few others the trap-nest record of the bird laying the dwarf egg was lost through some other slip. Since most of the dwarf eggs of known origin were produced each by a different individual, we shall arrive at the fairest estimation of the number of birds which produce dwarf eggs by considering that each of these 47 was laid by a different individual, and by one which had not produced one of the dwarf eggs of known origin—that is, we may consider that the 298 eggs collected were produced by 247 individuals. From the above considerations it appears that during the last eight years at the plant of the Maine Station 247 out of 4,800 birds, or 5.15 per cent, produced at least one dwarf egg.

By means of the data given by Warner and Kirkpatrick (26) we may also approximate the relative number of dwarf-egg producers among the birds in the third and fourth laying contest at Storrs, Conn. These birds also did not all have an equal chance, since the data were worked up after 7 of the 12 months of the fourth contest. During these contests 85 out of 1,820 birds, or 4.67 per cent, laid one or more dwarf eggs. If the data had been digested after the fourth contest had been completed, it is quite probable that a few more birds would have laid dwarf eggs—that is, the percentage given may be too low.

The close agreement of the two approximations indicates that about 5 per cent of the birds in an average flock will produce at least one dwarf egg.

VI.—SEASONAL FREQUENCY OF DWARF EGGS COMPARED TO NORMAL EGGS

Dwarf eggs are frequently found by poultrymen during the spring and early summer and somewhat less frequently at other seasons. During the eight years that these eggs have been collected at the plant of the Maine Station they have occurred during every one of the 12 months. However, 70.8 per cent of them were laid during the five months from March 1 to July 31. During some years more than 80 per cent were produced during these months. Table XXIII gives the number of dwarf eggs produced each month for each of the eight years. It gives also the percentage of all of the dwarf eggs which were produced during each calendar month and the monthly percentage of the annual egg yield as determined by Pearl and Surface (19) for the years 1899 to 1907.

TABLE XXIII.—Number of dwarf eggs recorded each month from February 1, 1908, to February 1, 1916, and the percentage of the total number of dwarf (1908-1916) and normal (1899-1907) produced during each calendar month

Year.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Total.
1907 and 1908..						1	1	5	5	4	0	0	<sup>a</sup> 16
1908 and 1909..	0	0	0	0	0	3	7	5	1	3	0	1	20
1909 and 1910..	2	1	1	0	0	3	7	5	5	0	10	0	34
1910 and 1911..	0	1	2	3	1	2	3	3	12	6	4	6	43
1911 and 1912..	2	1	2	2	2	0	7	7	10	17	2	7	59
1912 and 1913..	1	1	1	1	3	0	1	0	7	1	1	0	17
1913 and 1914..	0	3	0	0	2	0	4	8	4	5	1	0	27
1914 and 1915..	1	0	6	3	4	3	5	8	7	10	20	5	72
1915 and 1916..	0	5	2	2	<sup>b</sup> 1								<sup>a</sup> 10
Total...	6	12	14	11	13	12	35	41	51	46	38	19	298
Percentage of total number produced during month..	2.01	4.03	4.71	3.69	4.36	4.03	11.74	13.76	17.11	15.44	12.76	6.36	100
Percentage of total annual yield of normal eggs produced during month (1899-1907) .	<sup>c</sup> 6.36	<sup>c</sup> 4.27	<sup>c</sup> 3.59	<sup>c</sup> 6.91	<sup>c</sup> 9.08	<sup>c</sup> 8.44	<sup>c</sup> 12.50	<sup>c</sup> 12.30	<sup>c</sup> 10.80	<sup>c</sup> 9.67	<sup>c</sup> 8.44	<sup>c</sup> 7.64	100

<sup>a</sup> Years incomplete.

<sup>b</sup> Calculations in earlier parts of paper were completed before this egg was laid.

<sup>c</sup> These should follow August, as they are for the end and not the beginning of the year.

The more frequent occurrence of dwarf eggs during the spring and summer is seen either in the record for each year or in the sums at the foot of Table XXIII. It must be kept in mind that this is the natural breeding season of the fowl and that the total number of eggs laid during these months is greater than during the other months of the year. Whether or not the number of dwarf eggs in the breeding season is greater than is to be

expected if they occur in a given ratio to normal eggs can only be decided by a comparison of the production of dwarf eggs with the normal-egg production.

The monthly distribution of normal-egg production has been investigated thoroughly in the Maine Station flock by Pearl and Surface (19). Their investigations cover the eight years preceding the beginning of the present study. They summarized their data for the whole period by obtaining the percentage of the total yearly egg production which occurred during each month. This egg-production polygon may be used as a basis for a rough comparison between the relative seasonal frequency of dwarf and normal eggs. Figure 1 shows this egg-production polygon and a similar polygon showing for the eight years of the present investigation

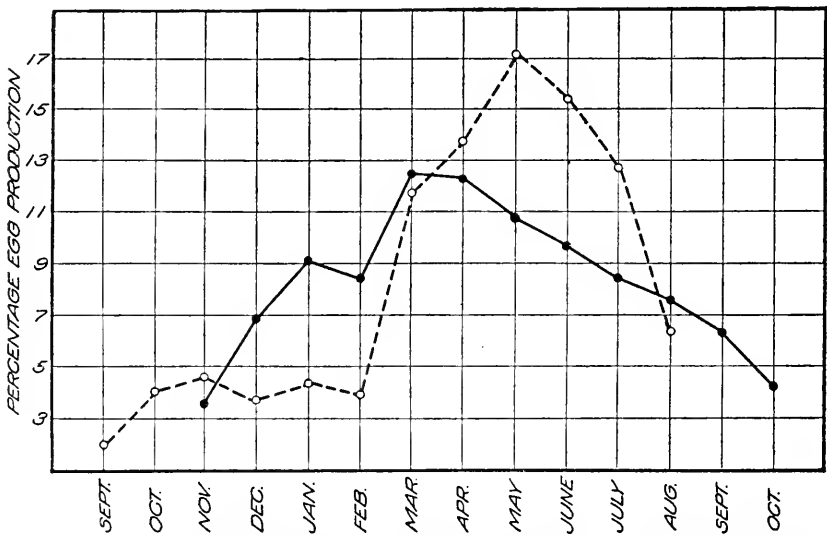


FIG. 1.—Diagram showing the percentage of the yearly total egg production (8-year average, 1899-1907) and the percentage of the total dwarf-egg production (8-year average, 1908-1916) which occurred during each month. Solid line=percentage of annual egg production. Dash line=percentage of annual dwarf-egg production.

the percentage of all of the dwarf eggs which were produced during each month. The data are given in the last two lines of Table XXIII. It will be noted that the two polygons do not begin or end with the same month. The reason for this is that the first set of data was collected for September and October after the birds were a year old, while, as already stated, during the period covered by the second investigation the data from September 1 to September 1 represent more nearly the data on a single group of birds.

From the diagram it is seen, as would be expected on the theory of chance, that during the months of heaviest normal-egg production more dwarf eggs are produced than at other seasons. Yet it is also seen that the two curves are by no means parallel. The egg-production curve rises

gradually through the fall and winter to its spring maximum and then drops away even more gradually. The dwarf-egg production curve does not rise during the fall and winter, but rises very abruptly during the spring to its maximum, which is three months later than the maximum for the normal-egg curve. It remains relatively higher than the normal curve through the early summer.

Since the data for the two polygons are derived from entirely different birds, it is desirable to pursue the investigations further and compare the

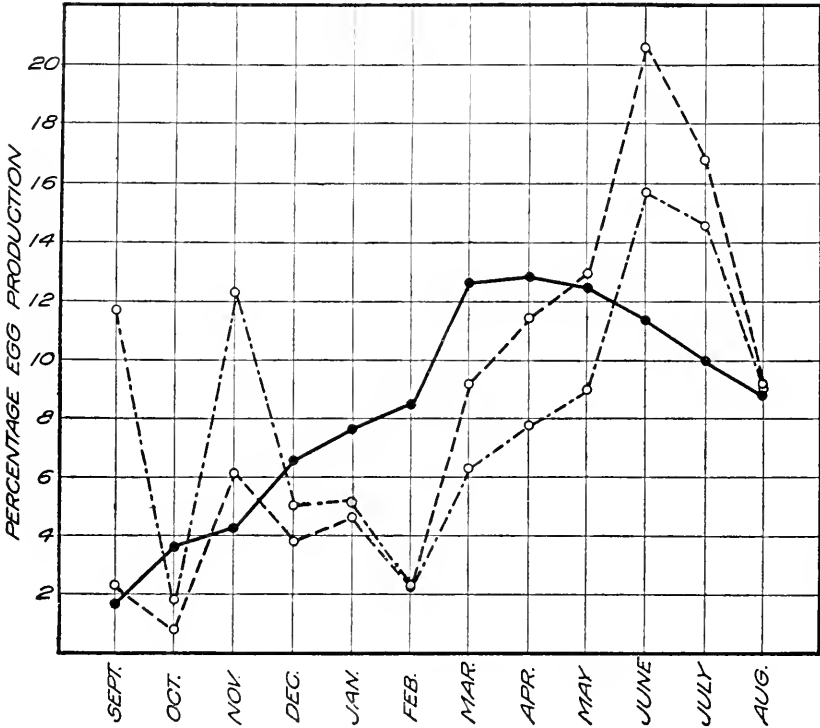


FIG. 2.—Diagram showing for the years 1911-12 and 1914-15 combined the percentage of the yearly total egg production and dwarf-egg production which occurred during each month and 100 times the percentage of the eggs produced each month which were dwarf. Solid line=percentage of total yearly egg production per month. Dash line=percentage of total yearly dwarf-egg production per month. Dot-dash line=the percentage ( $\times 100$ ) of dwarf eggs produced during the month.

number of dwarf eggs and the number of normal eggs produced by the same birds. The two years of maximum dwarf-egg production, 1911-12 and 1914-15, were selected for this study. The data for this study are given in Table XXII, which shows the total egg production, the dwarf-egg production, and the number of dwarf eggs per 10,000 eggs for each month of the two years. The last five columns of the table give the data for the two years combined. The summary data given in the last three columns are shown graphically in figure 2.

An examination of the diagram or of the data given in Table XXII shows that not only is the actual number of dwarf eggs smallest during the winter but that the number of dwarf eggs per 10,000 is also smallest. The irregular fluctuations of the fall are due to the fact that three of the abnormal birds already referred to laid during these months. The small number of normal eggs produced at this season gives great weight to these dwarf eggs in calculating the number of dwarf eggs per 10,000. Both the actual number of dwarf eggs and number per 10,000 increases through the spring, reaching its maximum in early summer some months later than the maximum for normal-egg production. It is thus shown that the dwarf-egg production is actually highest and also highest in proportion to the normal-egg production during the spring and early summer.

It thus seems probable that the disturbances in physiology which result in the production of dwarf eggs become more frequent with the onset of the natural breeding season and continue to increase in frequency during this season. The probable nature of these disturbances will be discussed later.

VII.—DWARF EGG PRODUCTION BY BIRDS WITH NORMAL AND WITH PATHOLOGICAL OVIDUCTS

The production of a dwarf egg is usually an isolated phenomenon—that is, a bird usually produces only one such egg. This fact, which has already been noted, is easily seen from Table XXIV.

TABLE XXIV.—*Number of dwarf eggs laid by each bird which produced one or more such eggs*

Number of dwarf eggs laid by a bird.	Number of birds.	Percentage of birds.	Number of eggs.
1.....	178	89.0	178
2.....	15	7.5	30
3.....	3	1.5	9
4.....	1	0.5	4
5.....	1	0.5	5
8.....	1	0.5	8
17.....	1	0.5	17
Total.....	200	100.0	251
Number of dwarf eggs laid by birds whose number was not known—that is, mostly floor eggs.....			47
Total.....			298

From Table XXIV we see that of the 200 birds which produced one or more dwarf eggs, 178, or 89.0 per cent, produced only one; 15, or 7.5 per cent, produced two; and only 7, or 3.5 per cent, more than two. The figures given by Warner and Kirkpatrick (26) for the birds in the Connecticut Station laying contest show an even larger percentage (94.11 per cent) of the dwarf-egg producers which lay only one dwarf egg. One

bird laid 14 dwarf eggs and no normal eggs. Each of the four others (4.71 per cent) laid two. It is thus apparent that the production of dwarf eggs is not usually an evidence of a permanent abnormality or derangement of the reproductive organs. This view is strengthened by a study of the egg records for the birds which produced dwarf eggs. In almost all cases these birds have a normal egg record. The dwarf egg is preceded and followed by normal eggs quite as though it was a normal egg. Autopsies were performed on several such birds, some immediately after the production of the dwarf egg. The sex organs were morphologically normal. There were, however, 11 of the 200 which showed evidence of a permanent disturbance, since few or no normal eggs were produced after the dwarf egg or eggs. In most of these cases the bird made nesting records. It has been shown by the authors (6,13) that "nesting records are, in the great majority of cases, at least, associated with ovulation into the body cavity or the backing into it of partly or fully formed eggs." Furthermore, autopsies were made on 5 of the 11 cases and all of these showed pathological conditions of the oviduct which would interfere with the passage of the egg but which did not entirely close the duct. These cases will be discussed in detail later. The point with which we are at present concerned is that the records for only 11 (5.5 per cent) of the 200 birds showed evidence of a permanent disturbance of the egg-forming processes. It is then evident that the disturbance which causes the production of a dwarf egg is usually of an accidental or at least temporary nature. However, there are certain pathological conditions of the oviduct which result in the formation of a dwarf egg instead of a normal egg.

The 11 cases where dwarf egg production appeared to be related to a permanent disturbance of the physiology of the sex organs include all of cases where the bird produced more than three dwarf eggs, two that produced three, one that produced two, and four that produced only one dwarf egg. The production of a succession of dwarf eggs or of a long series of nesting records with one or two dwarf eggs should lead one to suspect a serious disturbance of the oviduct.

We will first consider dwarf-egg production which is not associated with a morphological abnormality of the sex organs and will then discuss the pathological cases.

#### VIII.—THE RELATION OF DWARF-EGG PRODUCTION BY NORMAL BIRDS TO THE AGE OF THE BIRD AND TO THE POSITION OF THE EGG IN THE LITTER AND CLUTCH

##### A.—AGE

Attention has already been called to the fact that while dwarf eggs may be produced at any season of the year the spring breeding season, the season for highest normal-egg production, is also the season for highest

dwarf-egg production, both in actual number of dwarf eggs and in the proportion of dwarf to normal eggs produced. It was, however, shown that the maximum dwarf-egg production (either absolute or relative) did not coincide with the maximum normal-egg production.

Earlier studies (5) have shown a decided relation between the age of the bird and the tendency to produce multiple-yolked eggs—that is, birds are more likely to produce double- or triple-yolked eggs before they are entirely mature than later in life. In this connection it seemed worth while to investigate a possible relation between dwarf-egg production and age.

There were 189 normal<sup>1</sup> birds which laid one or more dwarf eggs. These birds laid 205 dwarf eggs. The age of the bird at the time the dwarf egg was laid could be determined in 202 cases. The age frequency distribution is given below.

Age in days.	Dwarf-egg frequency.	Age in days.	Dwarf-egg frequency.
150-209 . . . . .	11	690-749 . . . . .	6
210-269 . . . . .	14	750-809 . . . . .	2
270-329 . . . . .	22	810-869 . . . . .	2
330-389 . . . . .	65	870-929 . . . . .	0
390-449 . . . . .	52	930-989 . . . . .	0
450-509 . . . . .	19	990-1,049 . . . . .	1
510-569 . . . . .	4	1,050-1,109 . . . . .	1
570-629 . . . . .	0		
630-689 . . . . .	3		
			202

The constants calculated from this frequency distribution are: Mean =  $396.53 \pm 6.43$  days; standard deviation =  $135.57 \pm 4.55$  days; and coefficient of variation  $34.19 \pm 1.27$ . These constants must not, however, be accepted as a description of the age variation. It has already been noted that a large proportion of the birds are disposed of at the end of their first laying year—that is, when they are 15 to 17 months of age. There were, therefore, many more chances for a bird to lay a dwarf egg during her first year than later in life. From data in hand it is not possible to decide whether or not a bird is more likely to lay a dwarf egg during the second or third year than during the pullet year. The flocks were not depleted, however, except by the normal small mortality from natural causes, until the end of the first laying year. It may be noted from the distribution that pullets are increasingly likely to lay dwarf eggs up to the time they are 1 year old and that the chances then decrease up to the end of the pullet year. The mean age for dwarf-egg production among pullets may be calculated from the above distribution as far as and including the 450-509-day group. This mean is  $361.96 \pm 3.75$  days, approximately 1 year. It is apparent also that the second year maximum falls in the 690-749-day group—that is,

<sup>1</sup> That is, a complete study of their records, checked in many cases by post-mortem examinations, showed no abnormality.



when the bird is approximately 2 years old. Dwarf eggs are also produced by birds approximately 3 years old. From these data we see that dwarf egg production, unlike multiple-yolked-egg production, is not associated with immaturity of the bird, but that it is most likely to occur during the height of the breeding seasons in the successive years. These are, of course, the seasons of highest normal-egg production. In the case of a very few of the young birds and in an appreciable percentage of the old birds this is the only season in which the birds are in laying condition.

#### B.—POSITION IN THE LITTER

There is a widespread popular belief that a dwarf egg marks the end of a laying period or litter. This belief has found frequent expression in the literature from an early period to the present day. König-Warthausen (7) summarizes the belief of Tiedemann (25) as follows: "Er hält die dotterlosen Zwergeier für 'Reste von in Eileiter abgesondertem Eiweiss und Kalkerde' nachdem durch Jahreszeit oder Alter das Legen zu Ende ist." To this, however, he adds his own observation, "dass solche Fehlgeburten vielfach bei erstlegendern Hühnern (in meiner Sammlung aus Marz, April, und Mai) stattfinden." Wright (28, p. 579), in his discussion of normal eggs, says: "Of the other kinds of abnormal eggs the very small ones only containing albumen usually occur at or near the end of a batch of eggs." That this relation of the occurrence of a dwarf egg to a particular position in the litter is still somewhat generally accepted is shown by two recent statements. Lewis (9) says that "extremely small eggs are common at the beginning and end of a laying period." The second statement referred to occurs in an unsigned article on "Xenia in fowls" in the *Journal of Heredity* (29) and is as follows:

Experiments during recent years show that the eggs of any individual hen tend to become a little smaller as she approaches the end of her laying period, and the last one, it is generally believed, is likely to be a dwarf.

Since both dwarf eggs and broody hens are most common during the breeding season, it is not unnatural that a relationship between the two is assumed by poultrymen who do not trap-nest their birds. The use of the trap nest, however, soon dispels this illusion. Pearl, Surface, and Curtis (21) say that "the laying of one of these eggs is popularly supposed to mark the end of a laying period. This belief is without foundation in fact. They may be produced at any time." Warner and Kirkpatrick (26) some years later arrived at the same conclusion after a study of the data collected during two laying contests at Storrs. They summarize their data on this point as follows:

It was found that only two eggs out of a total of 103 indicate a resting period after the production of a small egg. In every other case the small egg was found in an

almost uninterrupted series of normal eggs. This seems to prove conclusively that small eggs may be laid at any time during a hen's laying period and that most small eggs are laid while hens are at the height of production.

The data used in the present investigation confirm the main part of this statement—that is, dwarf eggs may be produced at any time during the laying period. Our figures do not show that they are less likely to be produced at either end of the period than during its midst, as the above authors seem to imply by their statement that “most small eggs are laid while hens are at the height of production.” It is quite possible that they do not intend to make such an inference. Their records show that out of 103 eggs 7 were laid after a resting period of 14 to 25 days and 2 were followed by such a resting period. Our own records for normal birds which produced dwarf eggs and which completed the period of production during which the dwarf egg was laid show that out of 183 dwarf eggs 8 were first and 11 last eggs in their respective litters. A further analysis of our data on the position of the dwarf egg in the litter follows.

A few birds lay practically continuously from the beginning of laying until the first molt. Usually, however, there are well-defined laying periods which alternate with periods of nonproduction. The periods of production vary in extreme cases from two weeks to several months. In the present investigation any period of practically continuous laying, whatever its length, is considered a litter. In order to determine the relation of the production of a dwarf egg to its position in the litter, it is necessary to standardize the litter for the purpose of summarizing the data from the different cases. If the ordinal number of the day in the production period be divided by the whole number of days in the period, the resulting fraction will represent the position in the litter of an egg produced on that day. By this method the litter position of each dwarf egg produced by a normal bird which completed the litter was obtained. The frequency distribution for litter position of dwarf eggs is given below.

Fraction of litter.	Dwarf-egg frequency.
0        -0.099.....	26
.100- .199.....	19
.200- .299.....	10
.300- .399.....	16
.400- .499.....	13
.500- .599.....	21
.600- .699.....	24
.700- .799.....	15
.800- .899.....	10
.900- .999.....	29

183

Mean =                   0.506 ± 0.015

Standard deviation = 0.307 ± 0.011

This distribution is shown graphically in figure 3. It does not show a tendency of a dwarf egg to be produced at any particular position in the litter—that is, the variation in the class frequencies are irregular. The dash line in the figure represents the mean class frequency; in other words, it represents graphically the frequency distribution for 183 observations evenly distributed among 10 classes. It is the ideal distribution of things equally likely to fall into any one of the 10 classes.<sup>1</sup> The question which concerns us is whether or not the actual distribution differs from this ideal distribution by an amount greater than we would expect if the differences are due entirely to errors in sampling.

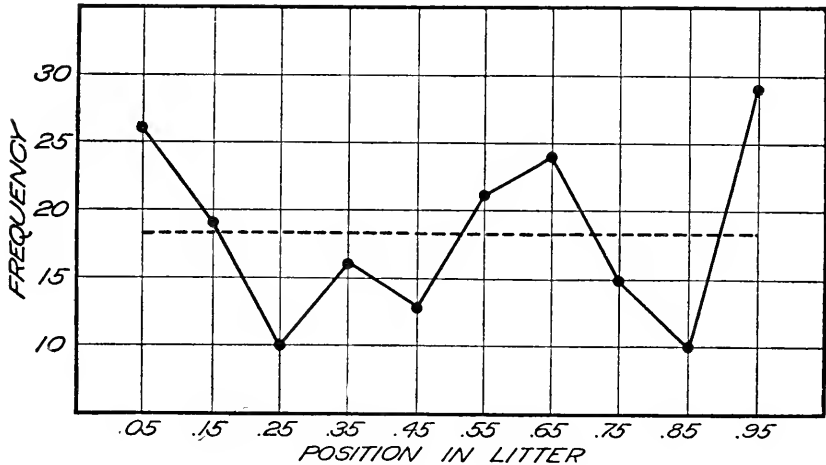


FIG. 3.—Diagram showing the number of dwarf eggs which occurred in each tenth of a litter. Dash line—the mean frequency.

A frequency curve of a given area is defined by its mean and the moments about this mean. The first four moments or the three constants, standard deviation,  $\beta_1$  and  $\beta_2$ , derived from these moments define a curve sufficiently for practical purposes. Two frequency curves of equal area which differ significantly from each other will show a significant difference between one or more of the similar constants. These constants and also the mean, which gives the location of the curve in space, were computed for both the actual and the ideal distribution. These constants, with their probable errors, and the difference between the similar constants for the two curves, with the probable error of difference, are given in Table XXV.

<sup>1</sup> A general treatment of this "horizontal line" frequency curve, which is a special case of Pearson's Type II, will shortly be published elsewhere.

TABLE XXV.—Mean standard deviation,  $\beta_1$ ,  $\beta_2$ , and the difference between the similar constants for the two distributions for the actual frequency distribution of the position of dwarf eggs in the litter and for an ideal evenly distributed frequency of the same size  $\alpha$

Distribution.	Mean.	Standard deviation.	$\beta_1$	$\beta_2$
Actual.....	0.506 ± .015	0.307 ± .011	0.003 ± .001	1.73 ± .05
Ideal.....	.500 ± .014	.287 ± .016	0	1.78 ± .06
Difference with probable error of difference.....	.006 ± .021	.020 ± .019	.003	.05 ± .08

<sup>a</sup> These constants are equal for any evenly distributed 10-class frequency with a class unit of 0.1, but the probable errors given in the table are calculated on the basis of 183 observations.

The last line of Table XXV shows that in no case does an essential constant for the actual curve differ from the similar constant for the ideal curve by an amount which is certainly significant—that is, the irregular fluctuations of the frequency curve for the litter position of dwarf eggs are not greater than the expected fluctuations of a random sample of the same size drawn from a population evenly distributed over the range. The present data, then, indicate that a dwarf egg is equally likely to occur at any time during a period of production.

#### C.—POSITION IN THE CLUTCH

A fowl seldom lays on every day during a litter. The actual time between successive eggs depends on the rate of fecundity of the individual at the time. This rate differs greatly with the individual and with the season of the year. It also, in general, increases from the beginning of a litter to a maximum and then decreases toward the end of the period of reproduction (4, 19). Since fecundity finds its manifestation in discrete units (eggs), the result of a very low rate is expressed by the production of an egg on a day preceded and followed by one to several days on which no egg is produced. A common low fecundity rhythm results in the production of an egg on every second day. More usually an egg is produced somewhat later on each of two or more successive days, and then a day follows on which no egg is produced. The next egg is produced early on the following day. The litter is thus objectively broken into a series of daily eggs, which we may call “clutches,” separated by one or more days on which no egg is produced. The size of a clutch varies from one egg to the extreme and unusual cases where a whole litter (sometimes of more than 40 eggs) is laid in a continuous daily series.

The general acceptance of the notion that a dwarf egg marks the end of a period of production suggests an investigation of the position of the dwarf egg within its clutch. In 197 of the cases where a normal bird produced a dwarf egg the bird completed the clutch to which the dwarf

egg belonged. Table XXVI gives for every size of clutch the frequency distribution of clutch position of dwarf eggs.

TABLE XXVI.—Clutch-position frequency of the dwarf eggs for every size of clutch

Number of eggs in the clutch.	Ordinal number of the egg in the clutch.															Total.	Percentage.
	1st.	2d.	3d.	4th.	5th.	6th.	7th.	8th.	9th.	10th.	11th.	12th.	13th.	14th.	15th.		
1.....	50	...	...	...	...	...	...	...	...	...	...	...	...	...	...	50	25.38
2.....	26	20	...	...	...	...	...	...	...	...	...	...	...	...	...	46	23.35
3.....	10	19	13	...	...	...	...	...	...	...	...	...	...	...	...	42	21.32
4.....	5	5	7	7	...	...	...	...	...	...	...	...	...	...	...	24	12.18
5.....	2	4	3	2	5	...	...	...	...	...	...	...	...	...	...	16	8.13
6.....	0	2	2	0	1	0	...	...	...	...	...	...	...	...	...	5	2.54
7.....	1	1	0	0	1	3	0	...	...	...	...	...	...	...	...	6	3.05
8.....	0	0	0	0	1	0	2	0	...	...	...	...	...	...	...	3	1.52
9.....	1	0	0	1	0	0	0	0	...	...	...	...	...	...	...	2	1.01
11.....	0	0	1	0	0	0	0	0	0	0	0	...	...	...	...	1	.51
15.....	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	2	1.01
Total.....	95	51	26	10	8	3	2	0	0	1	0	0	0	0	1	197	100.00

This table (XXVI) shows that 50 dwarf eggs occurred as 1-egg clutches—that is, no egg was produced on either the preceding or the following day. Forty-six occurred in 2-egg clutches, the other egg being in each case a normal egg. Of these, twenty-six were the first, and twenty the second, of the two eggs. Similarly through the table we may compare the number of dwarf eggs produced in the successive positions in a clutch of any given size. The clutches in which dwarf eggs occurred varied in size from one to fifteen eggs. A study of this table shows no apparent uniform tendency for a dwarf egg to occur in any particular position in a clutch.

In order to summarize the data for the various-sized clutches, it is necessary to standardize the clutch. A clutch may be conceived as a line of definite length. This line may be divided into as many segments as there are eggs in the clutch. Each segment may be assigned a value equal to the fraction which the distance from the origin to the midpoint of the segment is of the whole length of the line. An egg, then, has a definite clutch-position value expressed as a fraction of the clutch. These values are comparable for all sizes of clutches. For example, the value assigned to the middle egg of any clutch which contains an odd number of eggs is 0.500. A table was calculated which gives the value for each clutch position in each size of clutch. By means of this table the clutch position for each dwarf egg can be determined in terms which are comparable for all cases of dwarf-egg production whatever the size of the clutch.

The clutch-position frequency for the occurrence of dwarf eggs is given below.

Fraction of clutch.	Dwarf-egg frequency.
0. -0.199.....	19
.200-.399.....	40
.400-.599.....	25
.600-.799.....	35
.800-.999.....	28

147

Mean =  $0.518 \pm 0.015$

Standard deviation =  $0.267 \pm 0.011$

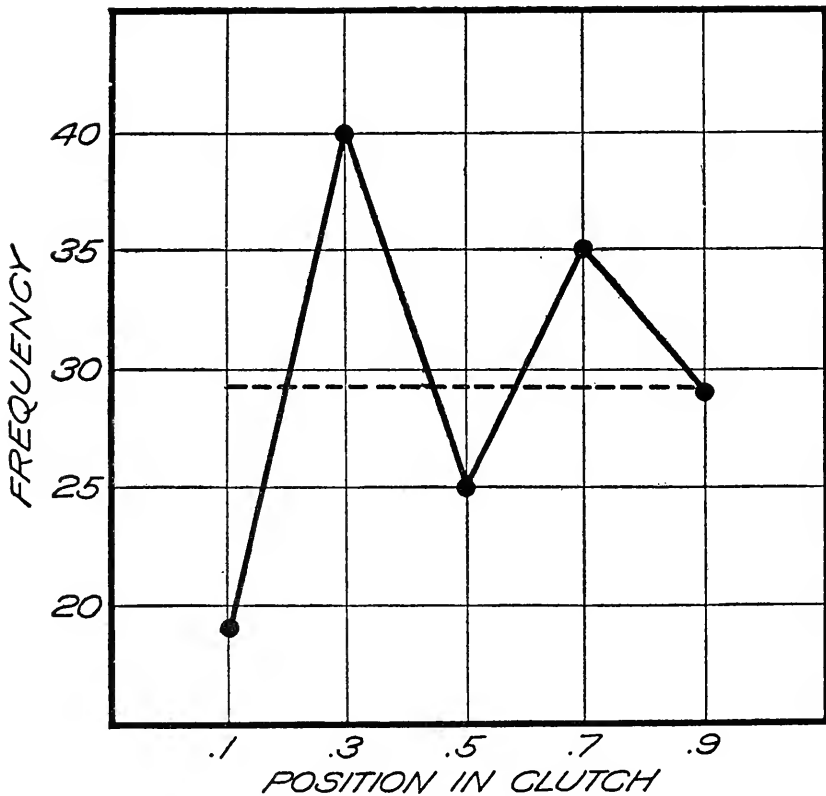


FIG. 4.—Diagram showing the number of dwarf eggs which occurred in each fifth of a litter. Dash line shows the mean frequency.

This distribution is shown graphically in figure 4.

There is no apparent relation of dwarf-egg production to any particular position in the clutch. The dash line in the figure, as in figure 3, represents an ideal uniform frequency for the same number of observations similarly grouped. In this case, as in the case of litter position, the actual frequency was tested against the ideal uniform frequency to determine whether or not the irregular fluctuations were greater than

would be expected from errors in sampling. The mean and the three constants which define frequency curves, standard deviation,  $\beta_1$  and  $\beta_2$ , were calculated for the actual and the ideal distribution. It has already been pointed out that two distributions with the same number of observations which differ significantly will show a significant difference between the values for one or more of these similar constants. The constants for each distribution with the differences between the similar constants in the two curves are given in Table XXVII.

TABLE XXVII.—Mean, standard deviation,  $\beta_1$ , and  $\beta_2$ , for the actual frequency distribution of the position of dwarf eggs in the clutch compared with the same constants for an equal even distribution with the same number of classes

Distribution.	Mean.	Standard deviation.	$\beta_1$	$\beta_2$
Actual.....	0.518 ± 0.015	0.267 ± 0.011	0.00005 ± 0.00003	1.740 ± 0.054
Ideal.....	.500 ± .016	.283 ± .011	0	1.700 ± .054
.Difference with probable error of difference...	.018 ± .022	.016 ± .016	.00005	.049 ± .076

The last line of Table XXVII shows that not one of these constants for the actual distribution differs significantly from the similar constant for the ideal distribution—that is, the irregular fluctuations in clutch position of dwarf eggs are not greater than would be expected to occur from errors of sampling. The present data indicate, then, that a dwarf egg is equally likely to occur in any clutch position.

IX.—PHYSIOLOGICAL CONDITIONS AND EFFECTIVE STIMULI WHICH LEAD TO DWARF-EGG PRODUCTION

It has been shown that dwarf eggs usually represent some temporary disturbance or some accident in the physiology of reproduction, since such eggs are preceded and followed by normal eggs. The disturbance is most likely to occur during the height of the breeding season, although it may happen at any time during the year. During any particular litter or clutch a dwarf egg is equally likely to occur at any time. Although the cause of dwarf-egg production is usually of a temporary character, there are cases where a bird lays only, or chiefly, dwarf eggs for some time. Other birds produce normal eggs for some time and then become habitual dwarf-egg producers. In the present section we will consider the nature of the disturbances, both temporary and permanent, which lead to the production of dwarf eggs.

Tiedemann (25) explained the origin of the dwarf eggs as the residue of albumen and shell secreted in the oviduct at the end of the laying. Wright (28) says that the occurrence of small abnormal eggs "need seldom

occasion anxiety. They usually occur at or near the end of a batch of eggs and merely show that the ovary is exhausting its supply of ova or yolks a little before the secreting parts of the oviduct are quite ready to suspend business." Lewis (8) explains dwarf-egg production, which he says is common at the beginning or end of a laying period, as "in part due to a diminution in the size, hence in the lessened secreting power of the oviduct." These views are untenable in the face of the facts cited above. Bonnet (2) says that such eggs mostly arise through pathological processes in the oviduct.

On the basis of unpublished data, Pearl, Surface, and Curtis (21, p. 176) made a statement of the factors which were probably involved in dwarf-egg production. The data on which this statement was based are included in the data used in the present investigations. The data then on hand indicated that three fundamental factors are concerned in dwarf-egg production. These are:

1. The bird must be in an active laying condition; the more pronounced the degree of physiological activity of the oviduct the more likely are these eggs to be produced.
2. There must be some foreign body, however minute, to serve as the stimulus which shall start the albumen glands secreting. This foreign body may be either a minute piece of hardened albumen, a bit of coagulated blood, a small piece of yolk which has escaped from a ruptured yolk, etc.
3. It seems likely, though this is a point not yet definitely settled, that ovulation—that is, the separation of a yolk from the ovary—must precede the secretion of albumen around the foreign body to form one of these eggs.

To a large extent the complete investigation confirms and extends these conclusions. The data which contribute to our knowledge of the physiology of dwarf-egg production are the complete egg records and the autopsy records of dwarf-egg producers.

#### A.—EVIDENCE FROM THE EGG RECORDS AND AUTOPSY RECORDS OF DWARF-EGG PRODUCERS WITH ABNORMAL SEX ORGANS

It has already been noted that the egg records for 11 of the 200 known dwarf-egg producers showed that few or no normal eggs were produced after the dwarf egg or eggs. Such birds usually make nesting records, the dwarf egg occurring in a series of the nesting records. As an illustration, the egg record of case 1 is given in Table XXVIII.

From this record it may be seen that the bird was a heavy layer, producing 162 eggs up to May 28. After this she produced only one normal egg (on June 26). The nesting records occurring in clutches indicate that the ovary passed through its normal cycles. Four dwarf eggs were produced in a series of nesting records. The bird made her last nesting record on January 16. Twenty-four days later (February 9) she was killed for data. She was in a normal healthy condition and was very fat. The visceral organs were apparently all perfectly normal, except the oviduct. The ovary contained an enlarging series of yellow yolks, four



of which were more than 1 cm. in diameter. There were no visible discharged follicles. The bird was evidently approaching another cycle of egg production. The oviduct was nearly the size of an oviduct in a laying bird of the same body weight. The organ had but one abnormality. Six cm. from the mouth of the funnel were two constrictions, separated by about 1 cm. of duct, with the same diameter as the rest of the albumen region. The finger could be pushed through these constrictions. There was no pathological appearance in the duct wall at these points. It seems probable, however, that these constrictions prevented the passage of the normal egg, but allowed the passage of a smaller body, as the beginning of the dwarf egg. No yolk was found in any of the dwarf eggs produced by this bird. The nucleus in each of three cases was one or more small lumps of coagulated albumen. The dwarf egg produced on November 21 contained a small-stalked hard-shelled dwarf egg. The entire egg weighed only 11.1 gm. Neither the outer nor inner egg contained any yolk.

TABLE XXVIII.—Egg record of case 1. <sup>a</sup>

Date.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Totals.
Sept.....																																3
Oct.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	27
Nov.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	23
Dec.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	21
Jan.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18
Feb.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	13
Mar.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	107
Apr.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18
May.....	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18
June.....	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	162
July.....	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	1
Aug.....	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	0
Sept.....	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	0
Oct.....																																0
Nov.....									n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	3
Dec.....									n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	0
Jan.....	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	0
Feb.....								D																								0

<sup>a</sup> "1" denotes a normal egg, "1-" a dwarf egg, "n" a visit to the trap nest but no egg, and "D" date killed for data.

Case 2 was that of another good layer which suffered a permanent disturbance, which hindered the production of normal eggs, but permitted dwarf-egg production. The case history of this bird follows. She began to lay on September 3, 1913. From this time until she stopped laying for the first molt, on October 5, 1914, she laid 218 eggs. On November 6, 1914, while the bird was still in nonlaying condition, three-fourths of her ovary was removed by a surgical operation. She began to lay again on December 29, 1914, and from this time until July 8, 1915, she produced 128 eggs. Three days later, July 11, she produced a dwarf egg. This was the first of a series of 7 dwarf eggs, the last of which was produced on July 23. Then followed an 8-day nonproductive period followed by a clutch of 3 normal eggs, on July 31 and Au-

gust 1 and 2. These were the last normal eggs produced. One more dwarf egg was produced on August 7. On August 29 the bird died of peritonitis.

At the autopsy a cylindrical dwarf egg was found in the oviduct. It was projecting from the isthmus into the shell gland. The egg had a shell which was thicker at the posterior end. Both the membrane and shell were incomplete at the anterior end. Several days before this bird died an egg similar to this one was found on the roosting boards of the pen in which she was kept. The funnel region of the oviduct was apparently normal. The glandular ridges were smeared over with what appeared to be albumen mixed with a small amount of yolk. At the anterior end of the albumen-secreting region the glandular ridges were very thick, and nodules of what appeared to be glandular tissue projected through the muscular layers. The region was somewhat contracted. Behind a narrow band of this tissue the duct was normal. In the body cavity free yolk was smeared over the intestines, and one yolk was walled off by peritoneum just below the ovary. Unfortunately no record was made of the contents of the egg found in the duct. Four of the eight dwarf eggs which were laid contained small drops or lumps of yolk. The other four were yolkless. All eight contained coagulation fibers which looked like normal chalazæ. The four yolkless ones contained no nucleus except these chalaza-like masses.

Apparently the ring of pathological tissue formed a partial constriction which hindered the passage of normal yolks. Yolks evidently entered the duct and were either extruded into the body cavity unbroken, or were broken and then entirely or mostly extruded. These yolks stimulated the secreting functions of the duct. In case all or most of the yolk was extruded the result was a dwarf egg. Three yolks evidently passed the obstruction unbroken and became the yolks of normal eggs.

Case 3 was that of a bird which was a fair producer during the early part of the season. She laid 150 eggs before July 1 and had always made some nesting records. The proportion of nesting records to eggs increased through the spring and early summer. In July there were as many nesting records as eggs. In August only two eggs were laid. The last of these, on August 27, was the last normal egg produced by this bird. From August 30 to October 16 the bird nested on every day except three. The only egg laid during this time was one dwarf, which was produced on September 16. The bird nested twice in November. There were two clutches of nesting records early in December. On December 13 the bird laid an egg which contained a normal yolk but which had a projection like a snail shell on the large end. The projection was formed of a membranous tube continuous with the egg membrane and filled with albumen. This tube was folded down onto the end of the other part of the egg and

was covered with a cap of shell. There was a distinct seam between the base of this cap and the shell which covered the rest of the egg, although they were continuous. Two days after this egg was laid the bird was killed, and an autopsy was performed. There was an egg in the oviduct just entering the isthmus. The lower end was covered with membrane. The upper end was prolonged into a string of albumen 5 or 6 mm. in diameter which extended 5 or 6 cm. up the duct. This egg then was similar to the egg laid two days previously in that it failed to round off normally at the anterior end. There was an abnormality of the oviduct which consisted in the presence of nodules of tissue in the glandular ridges of the funnel region. The nodules gave this lower portion of the funnel a quite abnormal appearance. Nodules were present in the peritoneum as well as in the oviduct. There were two large empty follicles on the ovary and a normal series of enlarging yolks, five of which were above 1 cm. in diameter. The other viscera were also normal. At the time of the autopsy the tumorous nodules in the lower funnel did not prevent the passage of yolks. The long series of nesting records at the time the dwarf egg was produced suggests that for a long period the pathological conditions of the duct may have prevented the passage of a normal yolk. The dwarf egg produced in the midst of this long series of nesting records contained some yolk wrapped in the chalazal fibers and some mixed with the thick albumen. The last normal egg had been laid 20 days before the dwarf egg. It seems therefore certain that the yolk in the dwarf egg was a part of a yolk which was broken either in the process of entering the duct or after it had entered. In the latter case the most of the yolk must have been extruded into the body cavity.

Another high producer which suffered a permanent disturbance which hindered normal-egg production was case 4. This bird produced 247 eggs during her first laying year. She had made occasional nesting records from the start, but the proportion of these to eggs increased markedly after July 1. There were, however, periods when the bird produced a litter of eggs without making nesting records. On February 13 of her record year the bird produced a dwarf egg. The egg which preceded this was a normal egg laid 13 days earlier. The dwarf egg contained chalaza-like coagulated albumen fibers, but no trace of yolk or other inclusion. Two days later the bird produced a normal egg. This was the last egg laid. Occasional nesting records followed. On May 5 (70 days after the last egg) the bird died and an autopsy was made. The ovary contained a normal series of enlarging yolks and four ruptured follicles. The body cavity contained a yellow fluid which was apparently a mixture of yolk and serum. A tumorous growth consisting of small solid tissue nodules was scattered all over the mesentery. A few nodules were present on the walls of the intestine. The upper half of the oviduct was badly diseased. The walls were thickened and hard. In places they were covered with large bunches of tumorous tissue.

This case again shows that a normal heavy-laying bird may develop a disease which affects the oviduct and prevents the passage of normal yolks, but which does not prevent the formation of yolks in the ovary. These yolks are ovulated into the body cavity. Since there was no yolk in the dwarf egg, it can not be proved that the egg formation was initiated by the entrance of a yolk which was later extruded. This may, however, have been the case. The occurrence of a normal egg only two days later shows that the ovary was in active condition. The immediate cessation of normal-egg production, the continued occasional occurrence of nesting records, and the condition of the ovary and oviduct at the autopsy strongly suggest that the passage through the duct was already considerably obstructed at the time the dwarf egg was produced.

The complete record of one more case (No. 5) is available. This bird did not begin to lay until November 13. She laid nearly continuously and made no nesting records until July 10. During this time (240 days) she produced 160 eggs. From July 10 to 23 the records show neither nesting nor eggs. This probably represents a normal period of non-production. No normal egg was produced by this bird after this period of nonproduction. On July 23 a dwarf egg was produced. This was followed by nesting records on the 24th and 27th. On the 31st another dwarf egg was produced. On August 3 and 4 the bird nested and on the 5th she produced a third dwarf egg. This was the last egg produced. From this time until the bird was killed (Sept. 2) nesting records continued to occur in series similar to the clutches of normal-egg production. We have no record or the contents of the dwarf egg produced on July 21. The eggs produced on July 23 and August 5 contained no yolk, but had as nuclei lumps of hardened albumen. The egg laid on August 5 was a dwarf egg which had a stalk attached to the large end. This stalk contained albumen and was covered with membrane and shell. To the lumps of albumen in this egg were attached long chalaza-like fibrous strings. One of these extended into the stalk. The autopsy record of this bird shows the ovary in a normal period of reproduction with a series of enlarging yolks, five of which were more than 1 cm. in diameter. There were four empty follicles visible. The anterior half of the oviduct was pathological. The walls were covered with a tumorous growth which appeared to be a proliferation of the muscular tissue. The outer layers of the walls of the intestine, portions of the oviduct ligament, and a small portion of the surface of the ovary contained small nodules of similar tissue. The body cavity contained a serous yellow liquid in which were lumps of yolk. The fact that the three dwarf eggs occurred between the production of the last normal egg and the complete cessation of egg production suggests that the disease may have gradually obstructed the passage through the duct. Whether or not the dwarf eggs were initiated by yolks which entered the duct and were later extruded can not be

decided, since they did not contain a trace of yolk. The continued occurrence of nesting records and the condition of the ovary at autopsy show that the reproductive cycles of the ovary were not interrupted. The dwarf eggs occurred during such a cycle.

The five cases of dwarf-egg producers cited above have several things in common: (1) Each bird was a normal, high-laying individual which became unable to produce normal eggs on account of a pathological condition of the oviduct. (2) In every case the part of the duct affected was the posterior end of the funnel, or the anterior end of the albumen-secreting region, or both. (3) The disturbance in each case was of a nature to constrict or prevent the normal expansion of the lumen of the duct. (4) In no case was the passage completely closed. (5) In each case there was convincing evidence that the ovary was in a normal reproductive cycle at the time the dwarf egg was produced.

Five of the sixteen<sup>1</sup> dwarf eggs produced by these birds contained as a nucleus a small quantity of yolk not inclosed in a vitelline membrane. This yolk was no doubt a part of a normal yolk, the rest of which was absorbed by the visceral peritoneum. Three of the five birds were absorbing yolk in this manner at the time of autopsy. The presence of a part of a yolk in the egg may have been due to any one of several causes. The three which seem most probable are the following:

1. A yolk may have been broken during its passage into the duct and only a part of it may have entered the duct.
2. A part of a yolk ovulated into the body cavity and, broken either before or after ovulation, may have been picked up by the funnel.
3. A normal yolk may have entered the duct and being unable to pass the pathological portion may have been broken and a part of it extruded into the body cavity. The remaining portion may have passed the obstruction, becoming the effective stimulus for the formation of the egg envelopes.

The effective stimulus in the case of the dwarf eggs which do not contain any yolk is difficult to ascertain. Some of these eggs contained what were apparently normal chalazæ. Most of them contained coagulated fibers which resembled the fibers of which chalazæ are formed. It is possible that in some or all of these cases a normal yolk has entered the duct, stimulated the upper duct to secrete chalazæ and some albumen, passed as far as the obstruction, and then been extruded, leaving behind sufficient chalazal material and albumen to furnish the mechanical stimulus necessary for the completion of the egg. Some of these eggs contained lumps of hardened albumen which may have arisen from albumen left in the duct or abnormally secreted. When the ovary is in a particular condition, such a mechanical stimulus may cause the secretion of the egg envelopes. It must be kept in mind, however, that a dwarf egg did not

<sup>1</sup> In two other cases the presence or absence of yolk was not recorded.

occur unless the ovary was actively producing yolks. In none of the above-mentioned cases was it impossible that a yolk had entered the duct and started the formation of the egg.

We have considered 5 of the 11 cases of dwarf-egg producers which were apparently permanently abnormal. Autopsies were not performed on the six other cases. The egg records for five of them (No. 6 to 10) resembled the egg records of the birds just discussed. No normal eggs were produced after the dwarf egg or eggs; also the birds made nesting records similar to egg records, indicating that the ovaries passed through normal reproductive cycles. The relation of the occurrence of dwarf eggs to normal eggs or nesting records was of a nature to show that they were produced only when the ovary was maturing yolks. Several of the dwarf eggs contained free yolk.

The record of the other bird (case 11) is worthy of special mention. This bird laid 17 dwarf eggs. These eggs were also produced when there was evidence that the ovary was in functional condition. The uniqueness of the case lies, first, in the unusual number of dwarf eggs, and second, in the fact that, although the number of dwarf eggs and nesting records and the proportion of these to normal eggs increased, some normal eggs were produced as long as there was any evidence that the bird's ovary was in laying condition. The egg record of this bird is given in Table XXIX.

TABLE XXIX.—Egg record of case <sup>a</sup>

Date.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	Totals.
Sept.....														n	n		I	I		n		n		I		I	I		I		6	
Oct.....	I		n		I	I		I		n		I		n			I		I		I		I		I		I		I		12	
Nov.....		n			I					I		I		I		I		n													6	
Dec.....													I	n		I	I		I		I		I		I		I		I		13	
Jan.....		I		I	I			I		I		I		I		I		I		I		I		I		I		I		I	13	
Feb.....																										I		I	I		I	4
Mar.....	I		I	I		I		I		n		I				I	I		I		I		I	I	I		I		I		53	
Apr.....	I		I	I		I	n	I		I		I		I		I	I	I	I		I		I	I	I		I		I		17	
May.....		I		I		I		I		I		I				I	n		n		I		n								8	
June.....	I		I		n		I			I		I		n	n		I	n	n	I		I		I		I		I		I	12	
July.....	I		n		n	n	n		I		I	n	I				n		n		n		n		n		n		n		4	
Aug.....	I					n	n		I			I	n		I	I		I	I		I						I		I		10	
Sept.....	I				n		n		n		n		n																		2	
Oct.....																															0	
Nov.....																															0	
Dec.....			I				I									I															3	
Jan.....																															0	
Feb.....																		S													0	

<sup>a</sup> "I" denotes a normal egg, "I—" a dwarf egg, "n" a visit to a trap but no egg, and "s" date sold alive.

A record was made of the contents of 16 of these dwarf eggs. Not one of these contained yolk, but all of them contained varying amounts of chalaza-like fibers, some resembling normal chalazæ. Thirteen contained no visible nuclei except the mass of coagulation fibers. One egg (laid on August 26) also contained a small lump of hardened albumen. The one laid on June 26 contained, beside the mass of chalazal fibers, a

small lump of tough membrane resembling shell membrane. The one laid on June 10 contained what appeared to be an empty yolk membrane. It will be shown later that a dwarf egg sometimes contains a ruptured yolk membrane from which most of the yolk has escaped, but this membrane contained no yolk. If it was a yolk membrane, all of the yolk had been squeezed out.

It is of some interest that this bird was a breeder, and the normal eggs laid between February 23 and April 22 were incubated. All but two were fertile, and 44 per cent hatched. It will also be noted that on August 26 both a dwarf egg and a normal egg were produced.

The dwarf egg and nesting records of this bird seem to indicate some disturbance of the morphology or physiology of the oviduct, which frequently but not always interfered with the entrance of a normal yolk or prevented its passage through the duct. Since this bird was sold alive, there is no record of the condition of the sex organs. As in other cases of dwarf eggs without yolk, it is impossible to tell whether the eggs were initiated by a yolk which entered the duct and was then extruded, or whether the fibers of chalazal material or other inclusions were efficient stimuli.

B.—EVIDENCE FROM THE EGG RECORDS AND AUTOPSY RECORDS OF NORMAL DWARF-EGG PRODUCERS ON WHICH AUTOPSIES WERE PERFORMED

Attention has already been called to the fact that, while occasional cases occur where dwarf-egg production is due to a permanent disturbance of the reproductive apparatus, it is in general not associated with such a condition. In fact, a dwarf egg may occur at any time in a clutch or litter, the production of normal eggs continuing as if the dwarf had been a normal egg. In these cases the egg records give no hint as to the reason for the production of a dwarf egg. Our only data are obtained from the contents of the egg and the autopsy examination of the reproductive organs. Such autopsy records are available for 27 of the 189 dwarf-egg producers, which were apparently normal at the time of the production of the dwarf egg. In 4 cases a dwarf egg was found in the oviduct or body cavity at autopsy. Only one of these birds had previously laid a dwarf egg. In 3 cases the bird was killed a few hours after the dwarf egg was laid. Autopsies were made on 20 other cases from 9 to 508 days after a dwarf egg was laid. While the general or permanent morphological condition of the sex organs of a dwarf-egg producer is shown by each of these records, the temporary condition of the organs at the time a dwarf egg is produced is shown only by the cases on which autopsies were made while a dwarf egg was in the duct or within a few hours after such an egg was laid.

At the time of autopsy the sex organs of the birds which had produced a dwarf egg from 9 to 508 days before death were in every stage of repro-

ductive activity from strictly nonlaying to fully functional condition. Eighteen of the twenty showed reproductive organs which were in every respect normal for their functional condition. Each of these birds had produced a dwarf egg<sup>1</sup> in a regular series of normal eggs and had continued to produce normal eggs in regular cycles. These birds either made no nesting records, or a few such records were scattered among normal eggs, as is frequently the case with normal birds which have never produced dwarf eggs. Whether or not these occasional nesting records indicate ovulations into the body cavity has never been investigated. Observations made on a large series of autopsies on laying birds indicate that ovulation into the body cavity is not a very rare accident in birds with sex organs which are morphologically normal.

In each of the other two cases also the dwarf egg was produced in a regular series of normal eggs and normal-egg production continued in regular series for months (case 19, nine months, and case 20, five months) after the dwarf egg had been produced. Case 19 never made a nesting record until eight months after the dwarf egg had been laid. She then made two and laid a litter of 11 eggs. These were the last eggs produced. During the next three months she showed no evidence of reproductive activity (neither eggs nor nesting records). She began the last week of her life with a series of four nesting records on successive days. Three days after the last of these she died of peritonitis. At the autopsy the ovary contained a series of seven enlarged but absorbing yolks and two empty follicles which could be certainly identified. The body cavity contained free decomposing yolk. The upper part of the oviduct was filled with an egg concrement composed of successive layers of coagulated albumen formed around a small tumor which was attached by a narrow neck to a glandular ridge in the albumen-secreting portion of the duct. This tumor was about the size of a normal yolk.

Case 20 continued to lay normally for five months after the dwarf egg had been laid. Three nesting records were distributed separately among 100 normal eggs. The bird then appeared to pass through a normal non-productive period of nine days. She then laid two eggs. A week later two clutches of nesting records occurred. During the next month she laid three eggs and made two nesting records. Occasional nesting records occurred during the next three months, but no more eggs were produced. The bird was then killed for data. At the autopsy the ovary contained a series of six enlarging yolks and three distinguishable follicles. The body cavity contained lumps of yolk. The funnel and the oviduct ligaments in the region of the funnel were pathological. They were red and fluted in appearance. The elongated lips of the funnel in the region of ligaments were fused together so that the opening of the funnel was no larger than the diameter of the tubular portion of the duct.

<sup>1</sup> In one case two and in another three at widely separated dates and each occurring in a series of normal eggs.



At the time of autopsy cases 19 and 20 showed a pathological condition of the oviduct which prevented the entrance of a normal yolk into the duct. The fact that these birds continued to be good layers for nine and five months, respectively, after the dwarf egg had been produced and then, by the cessation of normal-egg production and the occurrence of nesting records, showed a disturbance in their normal-egg production makes it seem probable that the pathological condition found at autopsy did not originate until some time after the dwarf egg had been produced. At least it is not safe to assert that a pathological condition of the duct existed at that time.

Of the seven cases of birds which either had a dwarf egg in the oviduct or body cavity at autopsy or had laid such an egg a few hours before death all had normal sex organs in fully functional condition. Each of these cases seems worthy of brief description. Case 21 was a late-hatched pullet which did not show any<sup>1</sup> reproductive activity until February. She then made a series of four nesting records and produced the dwarf egg as her first egg. The egg contained two small lumps of a dark, hard secretion and stringy albumen threads which looked like untwisted chalazæ. There was no yolk in this egg. The bird was killed a few hours after the egg was laid. She was in every way a normal healthy bird. The ovary contained a normal series of six enlarging yolks and four large and three small follicles. In the body cavity there were a few centimeters of a serous fluid containing yolk. In this fluid were found strings of tissue which may have been vitelline membrane. This pullet was then in full-laying condition; but for some reason not associated with an abnormality of the oviduct the yolks did not enter the duct, but were ovulated into the body cavity and absorbed. The origin of the stimulus which initiated the formation of the dwarf egg is not clear. While it is possible that all or part of a yolk entered the duct and was later extruded, there is no evidence for or against this view.

Case 22 was a normal pullet which had produced 48 normal eggs. She produced five normal eggs on successive days and on the sixth day produced the dwarf egg. The egg contained a lump or drop of yolk the size of a bean. The bird was killed a few hours after the egg was laid. The ovary contained a normal series of five enlarging yolks and four large and four small empty follicles. These follicles were all apparently normal and empty of yolk membranes. The body cavity contained a fluid which was partly yolk. In this case there can be no doubt that a normal yolk was ruptured either during ovulation or afterwards in the duct or body cavity and that a part entered or remained in the duct, forming the nucleus for the dwarf egg, while the rest was being absorbed by the visceral peritoneum.

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<sup>1</sup> A lone nesting record occurred in December, but the bird may have accidentally gone into a nest.

Case 23 had been laying normally. The dwarf egg was the third egg in a clutch. This egg contained a peculiar nucleus. It appeared to be a yolk membrane constricted in the middle. One half of this membrane was filled with yolk and the other half with a clear liquid resembling thin albumen. The bird was killed a few hours after this egg had been produced. The sex organs were in normal active condition. There was no yolk in the body cavity. Evidently the abnormal yolk was extruded from a follicle which presented no abnormal appearance after the yolk was ovulated. Since this yolk was much smaller than a normal yolk, it is probable that it was formed in one of the smaller follicles.

Case 29 was killed by the other birds. She had laid a dwarf egg four months earlier and had continued to lay until six days before death. At autopsy a membrane-covered dwarf egg was found in the body cavity. It contained a small amount of very light-colored yolk. The albumen was greenish. The largest empty follicle on the ovary was not more than 5 mm. in diameter. There were a few shiny granules on the peritoneal surface which appeared to be remnants of absorbing yolk. Apparently the dwarf egg had remained in the oviduct or body cavity for several days, as the yolk it contained must have come from a follicle which was nearly absorbed. In this case also a part of a yolk seems to have been the stimulus which initiated the formation of the dwarf egg, while the rest of the yolk was absorbed from the body cavity.

Case 25 was a bird which had been presented by Dr. Edith M. Patch to the Maine Agricultural Experiment Station for dissection. This bird was kept at the Station plant for a few weeks. During this time she laid several normal eggs, but her trap-nest record was not kept. When she was killed for dissection, her sex organs were in a normal active condition. The ovary had a regular series of enlarging yolks and four empty follicles, two of which were nearly full size. The isthmus of the oviduct contained a normal membrane-shelled egg. A small dwarf egg was found in the shell gland. This egg contained coagulated fibers of albumen which resembled untwisted chalazæ. There was no yolk or nucleus other than the coagulation fibers. No yolk was found in the body cavity. If in this case the small egg had been initiated by a yolk which was returned to the body cavity and absorbed, the small egg must have been in the duct long enough for the absorption to have been completed. The size of the follicles on the ovary made this seem improbable. The origin of the chalaza-like bunch of coagulation fibers is not known. It seemed probable that these were the efficient initiating stimulus which started the secretion of the rest of the egg.

Case 26 died from some unknown cause. In the shell gland a dwarf egg was found. This egg contained as a nucleus a small lump of hardened secretion the size of a pinhead. The sex organs were in a normal

active condition except that the five yolks on the ovary were beginning to be absorbed. There were two large empty follicles. (The bird had not laid for five days before death.) The body cavity contained free yolk. This bird had also ovulated into the body cavity and was absorbing the yolks. Whether or not any of the yolk had entered the oviduct and initiated the secretion and then been expelled is not known. None of it remained in the egg.

Case 27 was found dead where she had hung herself in a feed rack. A dwarf egg was found in the shell gland. This egg is shown in Plate CXIII, figure 1. It contained two drops of yolk surrounded by albumen, egg membranes, and a thin layer of shell. The body cavity contained a yellow liquid which seemed to be a mixture of yolk and serum. The oviduct was in a normal active condition.

The ovary contained a series of enlarging yolks and ruptured follicles. From the largest one of the latter yolk was dripping. On examination it was found that the stigma or rupture line of this follicle was forked at the end. The follicle had ruptured only along the two short arms of this forked line. The yolk membrane was broken, but remained within the follicle. An examination of the follicles which contained the growing ova showed that three out of four of these had forked rupture lines. The follicles removed from this ovary are shown in Plate CXIII, figure 1. The last four (*c, d, e, f*) show the follicles containing complete ova. Follicles *c, d,* and *f* have forked stigmata, while *e* has a normal straight stigma. Follicle *b* is the one which contained the ruptured and nearly empty yolk membrane. It can be seen from the illustration that the straight part of the stigma is unbroken, while the forked part is open. In this case it seems clear that the incomplete rupture of the follicle resulted in the bursting of the yolk membrane. A part of the yolk entering the duct furnished the stimulus for the formation of the dwarf egg. The rest of the yolk was being absorbed by the visceral peritoneum.

C.—EVIDENCE IN CASES WHERE A DWARF EGG FORMS A PART OF A COMPOUND OR A DOUBLE EGG

a.—COMPOUND EGG OF WHICH ONE PART IS A DWARF EGG

Recently an abnormal egg was produced by a bird in the Station flock, which gives additional evidence as to the physiological conditions and nature of the stimuli which may result in the production of a dwarf egg. The shell of this egg is shown in Plate CXIII, figure 2. This egg was compound, and the two parts were of quite unequal size. The component which filled the larger part of the shell contained a normal yolk in a normal membrane but there was a slight tear in this membrane, and free yolk was protruding from this tear. The hole which faced the small component egg was quite small, and little of the yolk had escaped.

This part of the egg had normal chalazæ and thick and thin albumen. The other part, which filled the small portion of the shell, contained a drop of free yolk surrounded by a thick albumen envelope, which was quite distinct from the albumen of the large part of the egg. No thin albumen was present in this part of the egg. The egg had been opened by cutting and lifting off an elliptical piece of the large part of the shell. When the egg was turned out through this opening, only the large part came out. It was then seen that an incomplete shell membrane separated the two components.

This egg is analogous to the type of double-yolked eggs where the doubleness is visible externally by a depressed ring around the shell, and where internally there is a fold of shell membrane projecting into the deepest part of the furrow. In such double-yolked eggs the thick albumens are entirely separate. It has been pointed out by Curtis (5) that such an egg must come about from the union of two eggs while the first egg is entering the isthmus, since the formation of the egg membrane is a discrete process taking place immediately when the egg passes the isthmus ring.<sup>1</sup>

The compound egg described above evidently represents the union of a dwarf and a nearly normal egg at this point in the duct. The point of peculiar interest is that the yolk for the two parts of the egg seems to have come from the same normal yolk. The fact that the small component is situated at the end which would have been the pointed end of the larger part had it formed a single egg suggests that the dwarf egg preceded the normal egg through the duct. It is conceivable that during ovulation the yolk membrane was slightly ruptured and that a drop of free yolk entered the duct ahead of the main body of the yolk. While this seems the most probable explanation of the phenomenon, the shape of the egg may have been modified by the presence of a dwarf egg following. In this case the yolk may have been ruptured either before or after ovulation and a drop left behind may have stimulated the formation of the dwarf egg.

The bird which produced this compound egg succumbed to roup four days after this egg was laid. She laid a normal egg the day before she died and at autopsy a normal, soft-shelled egg was found in the shell gland. The reproductive organs were in normal active condition.

Two other compound eggs where one component was a dwarf egg have been produced at the station plant. In neither of these cases was there any external evidence of doubling. The eggs were about as broad as the average egg of the individual, but were perceptibly longer (in one case 13 mm.), so that they appeared very long and narrow compared to the other eggs of the birds. There was also no folding in of the egg membrane

<sup>1</sup> The fact that when an egg is entering the isthmus as much and only as much of it as has passed in is covered with membrane was first noted by Coste in 1874, and has since been observed by many investigators, including the authors (15, p. 106).

between the two parts, and thin albumen surrounded both thick albumen envelopes, which were distinct. In both cases the dwarf egg was at the pointed end and the normal egg at the blunt, or air-cell, end. In both cases the membrane of the yolk in the normal egg was uninjured. In neither case was there any yolk in the dwarf egg. The only visible nucleus in each case was a mass of chalaza-like coagulated albumen fibers. In these cases also the dwarf egg seems to have preceded the normal egg down the oviduct. The normal egg apparently overtook the dwarf egg at the end of the albumen-secreting portion of the duct. The origin of the coagulation fibers, which apparently furnish the stimulus for the formation of the dwarf egg in these cases, is not known.

In one case the compound egg was produced by a pullet one month after she began to lay. During this month the bird had produced nine eggs and nested without laying on eight days. The bird nested without laying on the first, third, fourth, and fifth days before the abnormal egg was produced. The day following the abnormal egg she neither nested nor laid. On the next two days she laid normal eggs. From this time on the number of nesting records decreased and the number of eggs increased. This is the only abnormal egg ever produced by this bird. She continued to lay well until sold at the end of her pullet year.

In the other case the bird was about a year old. At the time the egg was produced she had been laying steadily for a month and a half. All the eggs had been normal. The bird had not laid on the day preceding the production of the compound egg. On the following day she produced a dwarf egg which contained a mass of chalaza-like coagulated albumen fibers, but no yolk. These two abnormal eggs were the first eggs in a clutch of five, the three others of which were normal. The bird continued to lay for  $4\frac{1}{2}$  months—that is, until the end of August—never again producing an abnormal egg. She was sold one week after she stopped laying.

*b.*—DOUBLE EGGS IN WHICH THE INCLOSED EGG, AND SOMETIMES ALSO THE INCLOSING EGG, WAS DWARF

A dwarf egg is sometimes inclosed within a normal egg, or may furnish the nucleus of a larger dwarf egg (10). The cases of this kind which have occurred at the Station plant will in the near future be described in connection with a discussion of double or inclosed eggs. So far as possible, the description of cases will be left to a future paper. It seems necessary to summarize them here. A dwarf egg may be returned up the duct and meeting a normal egg may be included with it in a common set of egg envelopes. Of more interest to the present investigation are the cases where a dwarf egg is inclosed in a larger dwarf egg.

One case where such an egg was produced by a bird with a constricted ring of tissue in the upper oviduct has already been cited. This egg was the first of a series of three dwarf eggs. (See Table XXVIII.) The

nucleus in each of the other cases was hardened secretion. The inclosed egg was a very small-stalked dwarf egg with a hard shell. There was no yolk in either the inner or outer egg.

An egg similar to the one just described but much larger (weight, 32 gm.) was produced by a 2½-year-old bird which had laid normally until the end of her second breeding season. She stopped laying about the middle of June and showed no evidence of reproductive activity until the middle of August. She then began making nesting records. On the 25th she produced the double dwarf egg. A week later she was sold. The inclosed egg was a hard-shelled, stalked, dwarf egg which weighed 7 gm. The end of the stalk was open. This egg contained a mass of chalazal fibers and thin albumen. The long axis of the inclosed egg lay in the long axis of the inclosing egg. Coagulated albumen fibers like untwisted chalazæ were attached to both ends of the egg. The mass at the closed end of the inner egg contained a small cluster of yolk granules and a small lump of hardened secretion. The outer egg had both thick and thin albumen, normal egg membrane, and hard shell.

Four other cases have occurred at the Station plant where a very small dwarf egg has been the nucleus for a larger dwarf egg. In none of these cases was there any yolk in the outer egg, although in two of them there was a small amount of yolk in the inner egg. In each case the dwarf egg was covered by an egg membrane without shell. Each of the outer eggs had normal egg membranes and shell. In three cases there were bunches of coagulated albumen fibers resembling chalazæ attached to the poles of the inclosed dwarf egg. In each case the bird producing the egg was a normal heavy-laying bird and the egg occurred in a normal clutch of from two to five eggs. In each case the double egg was the only abnormal egg ever produced by the bird.

It thus seems that in normal birds in active laying condition a dwarf egg may be forced up the duct and may furnish the stimulus for the formation of a set of egg envelopes in which it becomes inclosed.

#### D.—EVIDENCE FROM EGG RECORDS AND EGG CONTENTS

It has been shown above that in cases of dwarf-egg producers on which autopsies were made, both normal and abnormal birds produced dwarf eggs only when the ovary was in active condition. All cases on which autopsies have been made with a dwarf egg in the oviduct, or within a few hours after a dwarf egg was laid, showed large empty follicles. Every case but one showed also that a yolk had been ovulated at almost precisely the time the secretion of the egg envelopes of the dwarf egg began. In the other case the ovary contained a series of absorbing follicles, two of which were very large, indicating that both had been discharged within two or three days at most. One of these had furnished

the yolk present in the normal egg found in the isthmus. Since the egg record of the bird is not available, it is impossible to say whether the yolk discharged from the second large follicle had been contained in a normal egg laid within a day or two before death, or whether it had been absorbed with great rapidity from the body cavity. It has been shown by Pearl and Curtis (6, 16) that "yolks and partly or fully formed eggs may be absorbed rapidly and in large numbers from the peritoneal surface." Previous observations, however, would not lead us to expect that within the normal period of the formation of an egg in the duct the absorption of a yolk would be so complete that no trace of it could be found. It seems probable that the sex organs remained in a condition capable of response to a stimulus for egg production for a few hours after ovulation. The presence of two large follicles, however, shows that in this case also the sex organs were in the extreme of active condition.

In case an autopsy was not performed upon a bird which produced a dwarf egg the morphological condition and the physiological state of the sex organs at the time the dwarf egg was laid can be judged reasonably accurately by the egg record. In all cases not discussed under the section on abnormal physiological conditions associated with dwarf-egg production the dwarf egg was produced within a litter all the other eggs of which were normal. As already shown, the dwarf egg took any position in the clutch and litter. In all cases there was abundant evidence from the egg record that the sex organs were in active condition and were capable of producing normal eggs.

In the center of the thick albumen of every dwarf egg examined was found some firmer material. In a number of cases this firmer nucleus was simply a few coagulated threads of albumen which resembled the threads of a normal chalaza. Sometimes the mass of threads has the appearance of a normal chalaza, but more often it is an irregular mass of untwisted threads. Such a mass of threads, or one, rarely two, more or less perfect chalaza, is present in nearly all the dwarf eggs. Frequently it is accompanied by one or more small slightly reddish lumps which appear to be hardened albumen, or by small blood clots, or more frequently still by a drop or more of yolk. It has already been stated that more than half of all the dwarf eggs collected contained some yolk not in a yolk membrane. In these cases the yolk is frequently surrounded by a membrane-like layer of coagulated albumen fibers resembling a chalazal membrane. In many cases nearly normal chalazæ are attached. The contents of such an egg is shown in Plate CXIII, figure 3. In most of these cases there is no normal yolk membrane in the egg, but in a few cases the dwarf egg contained a ruptured normal yolk membrane from which most of the yolk had escaped. Beside these, each of a number of dwarf eggs contained a small yolk without a germ disk but inclosed in a complete vitelline membrane.

Table XXX gives the number and percentage of each kind of dwarf eggs classified as to the nature of the contained nucleus.

TABLE XXX.—*Dwarf eggs classified according to the nature of the contained nucleus*

Nature of nucleus.	Number of dwarf eggs.	Percentage of dwarf eggs.	Subtotals of percentages.
Drop of yolk, no yolk membrane.....	141	51.46	.....
Broken yolk membrane with some yolk.....	10	3.65	55.11
Small complete yolk.....	27	9.85	64.96
Chalazal threads with or without lumps of coagulated albumen or blood clots.....	96	35.04	100.00
Total.....	274	100.00	.....

From Table XXX we see that 55.11 per cent of all the dwarf eggs opened contained a portion of a yolk, and 3.65 per cent contained a broken yolk membrane. This fact, in connection with the autopsy records already discussed for birds killed while a dwarf egg was in the duct or immediately after one was laid, indicates that in at least 55 per cent of the cases the immediate stimulus to the active duct was a part of an egg yolk, the rest of which was absorbed from the visceral peritoneum. In case 27, discussed on page 1027, the vitelline membrane of the yolk which furnished the stimulus was still within its ovarian follicle, although part of the yolk was in the dwarf egg in the shell gland and most of the rest in the body cavity. In this case the yolk was broken during ovulation, and only a part of it entered the duct. In the other case it is impossible to tell whether the yolk was broken during or after ovulation. It is possible either that the yolk was ovulated into the body cavity and subsequently broken and a part taken up by the duct; or on the other hand, it may have entered the duct and later been broken and a large part of it expelled.

Parker (10) described an ovum in ovo where the inclosed egg was yolkless and the inclosing egg contained a little "yolk substance." He believed that this "yolk substance" was the remnant of a normal yolk which had been ruptured and most of which had escaped. This suggested to him the question, "Is it possible that the yolkless condition of the inclosed egg is also due to the loss of its yolk?" However, he believes the evidence convincing "that albumen can be formed by the oviduct without the presence of yolk."

In 9.85 per cent of the dwarf eggs the stimulus to the active duct was an abnormally small yolk which for some unknown reason was produced and ovulated by the ovary. These cases apparently differ from normal egg production only quantitatively—that is, in the size of the stimulating yolk.



It is seen from Table XXX that 64.96 per cent of all the dwarf eggs produced were apparently initiated by the presence of yolk in the duct.

The presence of almost normal chalazæ in a few of the eggs without yolk suggests that a yolk may sometimes enter the duct, stimulate secretion of chalazæ, and then be extruded, leaving behind enough chalazæ and albumen to furnish the necessary stimulation for the completion of the egg.

X.—RELATION OF DWARF-EGG PRODUCTION TO OTHER OBSERVED PHENOMENA OF EGG PRODUCTION WHICH OCCUR IN NATURE OR HAVE BEEN EXPERIMENTALLY PRODUCED AND THE CONTRIBUTION OF THIS STUDY TO OUR KNOWLEDGE OF THE NORMAL PHYSIOLOGY OF EGG PRODUCTION

It has already been noted that five of the six birds on which autopsies had been performed while an egg was in the oviduct or immediately after one was laid were absorbing yolk through the visceral peritoneum. In three cases the dwarf egg also contained yolk. In two of the other cases, however, no yolk was found in the dwarf egg, although the body cavity contained yolk. This suggested, first, that ovulation or a specific condition of the sex organs immediately accompanying it was the essential stimulus for the secretion of the egg envelopes by the duct; or, second, that such a specific condition being present, the secretion of the egg envelopes was stimulated by the small lump of hardened albumen, which in these cases seemed to be the nucleus of the dwarf egg; or, third, that a yolk had entered and then been expelled from the duct.

That neither ovulation nor any condition of the sex organs associated with it is alone sufficient to cause the formation of a dwarf egg is certain. Birds known to have ovulated into the body cavity for a long time, due either to a morphological, physiological (6), or surgical (16) disturbance, which prevented the yolk from entering the duct but did not otherwise disturb the mechanism, did not produce dwarf eggs. Some stimulus other than the condition of the sex organs is necessary to start the secreting activity of the duct. In normal eggs and in dwarf eggs with yolk this stimulus (mechanical or chemical) is furnished by the yolk.

The fact that all dwarf eggs without yolks contain some nucleus firmer than normal albumen, together with the fact that in one case where the bird had a dwarf egg with such a nucleus in the shell gland at autopsy no yolk was found in the body cavity, suggests that when the sex organs are maturing and ovulating successive yolks from the ovary a mechanical stimulus may initiate the secretion of the egg envelopes.

Experiments performed by Tarchanoff (24) and Weidenfeld (27) have shown that a complete set of egg envelopes may be formed around an artificial yolk. Tarchanoff used an amber bead and Weidenfeld used an artificial yolk of wood or rubber. The authors, using a glass marble or an artificial yolk of agar, have confirmed this result. The experiments

have been referred to by one of the authors (5), but have not been described in detail. One of these eggs is shown in Plate CXIII, figure 4, *a*. The agar artificial yolk which formed the nucleus of this egg is shown in *b* of the same figure. This artificial yolk, which weighed 4.32 gm., was inserted through a slit in the middle of the albumen-secreting region and pushed posterior to the slit. The duct was tied on each side of the slit. The morning after the operation the membrane-shelled egg, which weighed 14.06 gm., was found on the floor of the cage.

In another successful case a glass marble coated with vaseline was inserted into the funnel, and the funnel was then closed by sewing the lips together. On the day following the operation the bird laid a hard-shelled egg which weighed 36.17 gm. This egg contained a small lump of vaseline as a nucleus. Six days after the operation the bird died. At autopsy the marble was found caught in the thread that sewed the mouth of the funnel. In this case it was impossible for a yolk to enter the duct, since the funnel lips were sewed together. The stimulation must have come from the marble or the vaseline.

Tarchanoff (24) notes that he obtained this result in only 1 out of 11 cases. The authors obtained a perfect result in only 2 out of 12 trials. Three other results were partially positive. In one case the bird was killed 24 hours after the operation and the agar yolk was found in the upper isthmus covered with a thin layer of thick albumen. In two other cases, where the birds succumbed to postoperative peritonitis, the artificial yolk, surrounded by a thin layer of coagulated albumen, was found in the duct at autopsy. In the other seven cases the artificial yolk was either laid without egg envelopes or was found naked in the duct at autopsy. All the birds used in these experiments were in active laying condition at the time of the operation. Two to three weeks after the operation autopsies were performed on five of the seven birds giving negative results. In two of these the sex organs were in the state to be expected in a bird which had stopped laying two or three weeks previously and was not approaching a new laying period. In the three other cases the sex organs were in functional condition, but no empty follicles were found on the ovary. We have noted elsewhere (16) that "a bird is usually not in laying condition for some time after any serious abdominal operation involving prolonged anesthesia and considerable surgical shock." Sellheim (23) notes that after removal of the oviduct the ovary at first shrinks; but since it comes again into functional condition, he believes that the postoperative shrinking is due to the severe operation. It seems that in the negative and partly positive cases described above the general physiological disturbance due to the operation may have lowered the general tone of the organism, or possibly the specific tone of the reproductive apparatus, to a point where the duct was unable to respond to stimulation in its normal manner.

The results show conclusively that in a certain stage of activity the oviduct responds to a mechanical stimulus by the secretion of the egg envelopes.

The fact that in a bird approaching a period of laying the oviduct enlarges as the yolk enlarges has long been recognized. In a bird which has not laid for two or three months and is not preparing for another production period the sex organs are in strictly nonfunctional condition. The ovarian eggs are scarcely larger than a pinpoint. The oviduct is a small, almost straight thin-walled tube, weighing from 2 to 3 per cent of its weight when in functional condition. As the ovary approaches laying condition, the oviduct enlarges. When the first group of oocytes start on their final growth period, the increase in the size of the duct is perceptible. By the time the first yolk is mature, the oviduct is also normally in functional condition. That this correlation is entirely due to the ovary is shown by the fact that the removal of the oviduct has no influence on the development or functional activity of the ovary (23, 16). Normally the oviduct is in functional condition only while the ovary is maturing yolks. The correlation is now commonly attributed to the internal secretion of the ovary. Bartelmez (1) working on pigeons states that "interstitial cells of the ovary show much greater signs of activity in functioning ovaries than do those in ovaries of birds that have not laid for a long time." A fact cited by Pearl and Curtis (16) indicates that the connection is not nervous, or at least that it is not conveyed to the oviduct through the nerves. This fact is that after the removal of a large part of the oviduct any part not removed passes through growth and cyclic changes associated with the periods of ovarian yolk production, exactly as though the duct were intact. Observations made in connection with other researches have shown that enlargement of the oviduct is not necessarily connected with yolk formation, although this is the normal relation. The two classes of exceptions that have been noted are: First, certain hermaphrodite fowls have been observed (14) that have ovaries largely made up of stroma rich in connective tissue and containing no large follicles, and yet these birds had oviducts from one-half to three-fourths the size of a functional duct; and, second, birds with certain types of ovarian tumors, but without enlarging yolks, have been observed to have nearly functional-sized ducts.

These facts taken together indicate that the functional condition of the oviduct depends upon some substance formed in the ovary, usually at the time yolks are maturing, but in certain pathological cases at other times also. This substance is probably an internal secretion carried by the blood, since the ovary can cause the enlargement to functional size of a small piece of oviduct the normal nervous connections of which have been destroyed. The fact that dwarf eggs are produced only when the bird is maturing and ovulating yolks and the fact that more than 50

per cent of the trials to induce egg formation around artificial yolks were failures suggest that the sex organs must be and must remain in absolute functional condition until the egg is completed.

Loeb (9) showed that the mammalian uterus responds to a mechanical stimulus by the formation of the maternal placenta during a definite period after ovulation. He finds that during this period the uterus is sensitized by the internal secretion of the corpus luteum. We may conceive that the specific state of the oviduct in the fowl which renders it capable of responding to mechanical stimulation, be it yolk or foreign body, is associated with some quantitative or qualitative difference in the internal secretion of the ovary. While from the data given above it is possible that it is due to some postovulation change in the ovary, this seems improbable. We know that in many and probably in most cases in normal-egg production the duct responds to the first yolk of a series ovulated. This response occurs immediately after ovulation—that is, there is not sufficient time for a change in the internal secretion of the ovary occurring at or after ovulation to affect the state of the duct.

An observation made some time ago also has a bearing on this point. A bird which had laid three days earlier was selected for an abdominal operation. She was accidentally killed with an overdose of ether just after the incision was made. The oviduct did not contain an egg, but the funnel was in active motion when first observed. It responded quickly and sharply when stimulated by pinching with the forceps. The albumen region also responded to this stimulus by strong peristaltic movements. A 10-cm. piece from the albumen-secreting region of this very active duct was cut open lengthwise and spread out flat with the glandular surface exposed in a warm damp chamber moistened with salt solution. Small bits of cork were scattered on the surface in order to study ciliary motion. The ciliary activity continued for  $1\frac{1}{2}$  hours. At the end of this time it was noted that a very thin film of albumen was visible on the surface of the mucosa. In this case an isolated piece of oviduct responded to mechanical stimulation by the secretion of a very small amount of albumen. This duct had not been sensitized by an immediately preceding ovulation. The last ovulation had taken place four days before the bird was killed. The active movements of the funnel when the body cavity was opened suggested that an ovulation was about to take place. Either the duct had remained in a condition capable of a secretory response for four days or it had again come into such a condition with the maturing of another yolk.

The above-described experimental work and the observations on the conditions under which dwarf eggs are produced indicates that mechanical stimulation of the oviduct results in the formation of egg envelopes only under a particular condition of the duct which seems to be associated with the maturing of yolks by the ovary. The sensitization of duct, if this is the proper explanation of the phenomena observed,

apparently precedes ovulation. Further work is necessary, however, to determine the factors involved in the specific condition of the duct which causes it to respond to stimulation by the secretion of the egg envelopes.

It would seem from the above considerations that the presence in a completely functional oviduct of a small solid or semisolid substance capable of presenting a mechanical stimulation may cause the production of a dwarf egg. Nearly two-thirds of the dwarf eggs, however, are known to be initiated either by abnormally small yolks or by parts of broken yolks. Their production may be associated with an abnormal condition of the ovary or with pathological conditions of the duct, but even in these cases the result was due not to the abnormality per se but to the fact that this abnormality prevented the entrance of a normal yolk or obstructed its passage through the duct.

The mechanical stimulus need not begin at the funnel in order to be effective to the parts of the duct lower down. In Tarchanoff's case (24) and in one of our own cases of perfect egg formation around an artificial yolk, the yolk was inserted into the duct through a slit in the albumen portion, the duct being tied off above this point. Pearl and Surface (18) showed that a mechanical stimulation (in this case feces introduced by anastomosing the intestine to the side of the uterus) caused the formation of shell by the uterus.

The mechanical stimulation is of local character—that is, it is not transmitted down the duct for any measurable distance below the point where it is applied. Pearl and Curtis (16) have shown that “the stimulation of the advancing egg is necessary for the discharge of the secretion of the duct, since a duct closed at any level functions only to the point where the passage is interrupted.” In the cases of dwarf-egg producers with pathological ducts the abnormality of the duct was in each case of a nature to constrict but not close the lumen of the duct. Several eggs produced by these birds contained lumps of yolk, indicating that the nucleus of the egg had passed the constricted portion.

#### SUMMARY

(1) During the eight years from February 1, 1908, to February 1, 1916, 298 dwarf eggs are known to have been produced at the poultry plant of the Maine Experiment Station.

(2) During the two years of maximum dwarf-egg production the ratio of dwarf eggs to normal eggs was 1 dwarf egg to 1,158 normal eggs.

(3) Dwarf eggs are of two distinct types in respect to shape: First, the prolate-spheroidal type, and second, the cylindrical type.

(4) Dwarf eggs of the prolate-spheroidal type are much more frequently produced than the cylindrical type. In fact 95.4 per cent of the dwarf eggs studied were prolate spheroids.

(5) Dwarf eggs may also be classified according to the absence of yolk or its presence either as a small yolk in a yolk membrane or as free yolk.

(6) Of the 274 dwarf eggs opened 35.03 per cent were yolkless and 64.96 per cent, or nearly two-thirds, contained yolk. The yolk was inclosed in membrane in only 9.85 per cent of the dwarf eggs opened, while free yolk was present in 55.11 per cent of these eggs.

(7) Dwarf eggs with small yolks, while distinctly smaller than normal eggs, are significantly larger than dwarf eggs with little or no yolk.

(8) A comparison of the relative size of the several groups of dwarf eggs, normal eggs, double-yolked and triple-yolked eggs furnish a continuous line of evidence that the amount of albumen secreted depends to a large extent at least upon the degree of immediate stimulation due to the amount of yolk present.

(9) Although the evidence available is not sufficient for a positive statement, the shape of the cylindrical egg is probably due to the long form of the stimulating nucleus.

(10) Dwarf eggs with small yolks have indices which are higher than those for normal eggs and lower than those for other prolate-spheroidal dwarf eggs. This difference in index in the three groups is the reverse of their difference in size.

(11) This negative correlation between the shape, index, and size extends the evidence from former researches that the smaller the egg the broader it is in proportion to its length.

(12) Two factors may be involved in producing this negative correlation between shape index and size: First, the area of the glandular mucosa under stimulation at any one time must be related to the size, particularly the length, of the stimulating nucleus (yolk drop, normal yolk, or two or three yolks in tandem). Second, the oviduct, which is a tube with elastic walls, will offer more resistance to the passage of a large than a small body, and therefore when the plastic egg is forced through it by peristalsis it will exert a greater elongating pressure upon a large than a small egg.

(13) Dwarf eggs of each class are exceedingly variable when compared to normal eggs. This greater variation occurs in all the physical characters measured—that is, length, breadth, shape index, egg weight, yolk weight, shell weight, and possibly albumen weight.

(14) Dwarf eggs with small yolk resemble normal eggs in degree of variability as well as in size and shape more nearly than do other classes of dwarf eggs.

(15) The several size characters show different degrees of variation. They may be arranged from most to least variable as follows: Egg weight, length, and breadth. This arrangement is the same for dwarf and normal eggs.

(16) It is probable that the variation in yolk weight compared to the variation in the other egg parts and to the whole egg is greater in dwarf eggs with small yolks than in normal eggs.

(17) The interrelation of the size and shape characters in prolate-spheroidal<sup>1</sup> dwarf eggs of each class is as follows:

- a. Length and breadth, length and weight, and breadth and weight are significantly highly correlated in eggs of each group.
- b. Index and weight are negatively correlated. The correlation is significant for dwarf eggs with little or no yolk.
- c. In dwarf eggs with small yolks, yolk weight is highly correlated both with egg weight and with albumen weight.

The physiological significance of these correlations is discussed.

(18) During the last eight years 5.15 per cent of all the birds kept at the Maine Station plant are known to have produced at least one dwarf egg.

(19) Both the actual dwarf-egg production and the number of dwarf eggs per 1,000 eggs is lowest during the winter months. It increases through the spring, reaching a maximum in the early summer.

(20) In general the season of high normal-egg production is also the season for high dwarf-egg production both actual and relative to normal-egg production. The maximum of dwarf-egg production, however, occurs later in the season than the maximum normal-egg production.

(21) The production of a dwarf egg is usually an isolated phenomenon occurring only once or twice during the life of a bird. Only 3.5 per cent of the birds which produced one or more dwarf eggs produced more than two.

(22) A study of all the egg records and the available autopsy records for birds which produced one or more dwarf eggs shows that in most cases the disturbance which caused the production of the dwarf egg was of temporary character and was not correlated with a morphological disturbance of the sex organs.

(23) Eleven of the two hundred dwarf-egg producers, however, showed evidence that a permanent disturbance had occurred. In these cases few or no normal eggs were produced after the dwarf egg or eggs, although nesting records indicate that the ovary passed through normal reproductive cycles.

(24) Autopsies were made on five of these cases, and all of them showed some pathological condition of the oviduct which interfered with the passage of the egg, but did not entirely close the duct.

(25) In normal birds dwarf-egg production is most likely to occur during the height of the breeding season. It is not associated with immaturity of the sex organs.

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<sup>1</sup> The same relations apparently also hold for cylindrical dwarf eggs, but the number observed was too small to determine the degree of relationship.

(26) The popular notion that a dwarf egg marks the end of a period of production is without foundation. A dwarf egg is equally likely to occur at any time during a clutch or litter.

(27) A dwarf egg may be overtaken by a normal egg and form one of the components of a compound egg similar to a double-yolked egg except that one part is a dwarf egg.

(28) A dwarf egg after it has received its membrane or its membrane and shell may be returned up the duct and be included in the succeeding normal egg, or it may act as the stimulus for the formation of a larger inclosing dwarf egg.

(29) Dwarf eggs are produced only when the ovary is in the absolutely active condition associated with the maturing of yolks. This is true whether the bird has a normal or pathological oviduct.

(30) When the sex organs are in this condition, a mechanical stimulation of the oviduct by an artificial yolk may result in the formation of a complete set of egg envelopes.

(31) The mechanical stimulation need not begin at the funnel in order to be effective to the parts lower down.

(32) The mechanical stimulation is local in its effect—that is, it is not transmitted down the duct any distance below the point to which it is applied.

(33) Dwarf eggs may be and probably often are produced by the stimulation of an active duct by some material particle which is not yolk.

(34) At least 65 per cent of the dwarf eggs studied, however, were initiated by an abnormal small yolk or by a part of a normal yolk. Certainly in some and probably in all the latter cases the rest of the yolk was absorbed by the visceral peritoneum.

(35) Neither the absolute time relation between ovulation and the ability of the duct to respond to mechanical stimulation nor the nature of the connection between the state of the ovary and the duct is certainly known.

(36) It is suggested that the oviduct may be sensitized by some change in the internal secretion of the ovary associated with the maturation of yolks.

(37) It is also pointed out that if this is the case the change in the secretion probably precedes ovulation.

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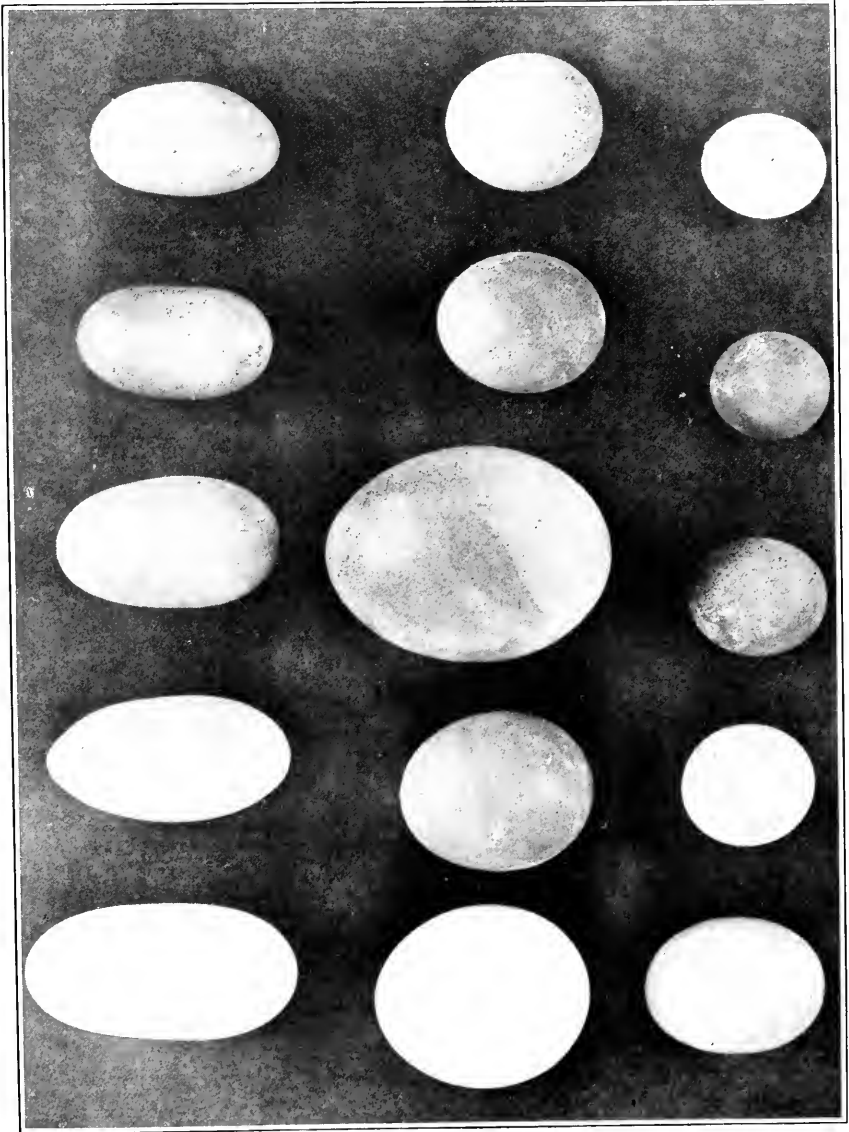
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PLATE CXII

A collection of dwarf eggs with a normal egg in the center of the group.  $\times 2/3$ .



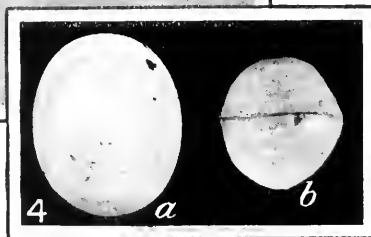
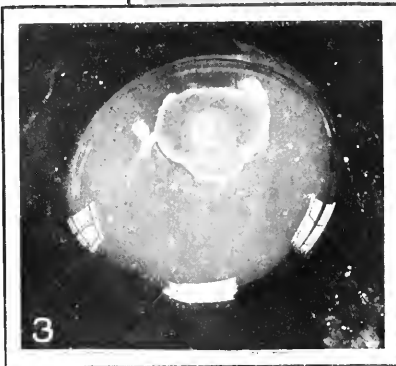
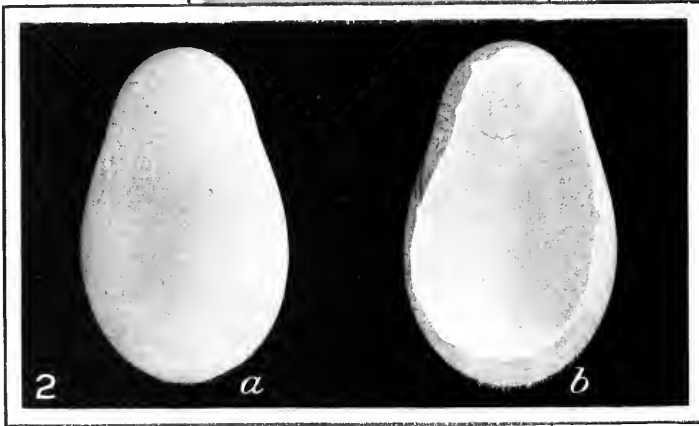
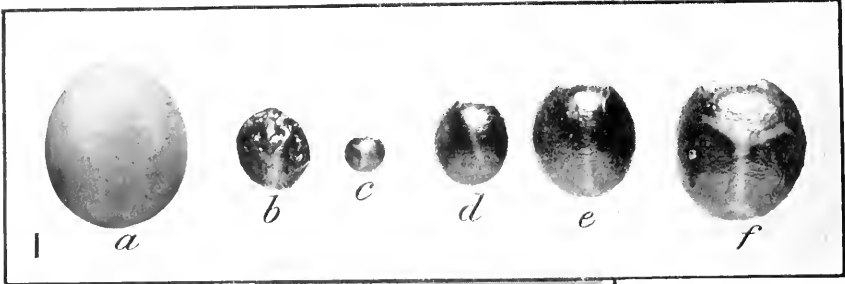


PLATE CXIII

Fig. 1.—Ovarian follicles (*b-f*) and the dwarf egg *a* from case 27. Follicle *e* has a normal straight stigma. Follicles *b*, *c*, *d*, and *f* have stigmata which are forked at the end. The forked portion of the stigma of *b* has ruptured; the yolk membrane is broken and most of the yolk has escaped. Part of the escaped yolk was in the body cavity and part formed the nucleus of the dwarf egg *a*.  $\times 2/3$ .

Fig. 2.—Shell of a compound egg which was composed of two albumen masses partly separated at the level of the seam in the shell by an incomplete egg membrane. The larger component contained a normal yolk with a slight puncture in the yolk membrane. The smaller one contained a drop of yolk which apparently came from the yolk in the other part. *a*, Outside view of shell; *b*, inside view.  $\times 2/3$ .

Fig. 3.—Dwarf egg containing a mass of yolk not in a yolk membrane, but separated from the albumen by a membrane-like layer of chalazal threads. Note nearly normal chalazæ.  $\times 2/3$ .

Fig. 4.—Dwarf egg formed around an artificial yolk of agar which was inserted into the oviduct. *a*, Complete egg; *b*, agar yolk.  $\times 2/3$ .





## $\alpha$ -CROTONIC ACID, A SOIL CONSTITUENT

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In a preliminary examination of a sample of Susquehanna fine sandy loam soil from Texas, which was made in October, 1915, by Dr. E. C. Shorey, who was at that time connected with the Office of Soil-Fertility Investigations, an unsaturated organic acid was isolated. In a subsequent examination of the same soil by the writers this compound was again isolated, and its identity with  $\alpha$ -crotonic acid has now been established.

The soil was taken from an infertile spot in a field near Marshall, Tex. The infertile spots, which are devoid of all vegetation, have been observed for three years in this locality, and the area of these spots is gradually increasing.

The soil in this district is described as a Susquehanna fine sandy loam, from 8 to 18 inches deep, with an average of about 14 inches (8).<sup>1</sup> The subsoil is a stiff clay of a red color or red mottled with yellow and gray extending to a depth of several feet. The color of the soil is prevailing gray, but for a few inches above the subsoil it has a reddish cast. Because of the impervious nature of the subsoil, the drainage is very poor, and special methods of soil management, with the object of producing better drainage, have been recommended and to a limited extent practiced. This soil is deficient in lime or other basic material and is very poorly drained. It has also been found to have a high reducing power and a rather low oxidizing power. It therefore seems to present optimum conditions for the formation and accumulation of organic acids.

In the isolation of  $\alpha$ -crotonic acid an alkaline extract was obtained by treating the soil with an aqueous 2 per cent sodium-hydroxid solution for 24 hours at room temperature. The extract was made slightly acid with sulphuric acid and filtered. The acid filtrate was then extracted with ether and the ether extract was evaporated to about 200 c. c. and shaken up with a concentrated solution of sodium bisulphite to remove aldehydes or other substances which combine with this reagent.

The bisulphite solution was drawn off and extracted several times with fresh ether. All of the ether extracts were then combined and slowly evaporated to a brown sirup in a small crystallizing dish. At this point the dish was covered with a watch glass containing ether and maintained at a low temperature on a steam bath. A white crystalline solid gradually sublimed on the watch glass. The sublimed substance was dried between filter paper and recrystallized from petroleum ether.

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<sup>1</sup> Reference is made by number to "Literature cited," p. 1045.

The substance was further purified by subliming several times at a low temperature and was dried over anhydrous calcium chlorid.

The properties of the substance thus obtained were found to be identical with those of  $\alpha$ -crotonic acid. The purified soil substance melts at  $72^{\circ}$  C., while  $\alpha$ -crotonic acid melts at  $72^{\circ}$ . A mixture of Kahlbaum's chemically pure  $\alpha$ -crotonic acid (further purified by sublimation) and the soil compound melted at  $72^{\circ}$ .<sup>1</sup>

The purified soil compound is soluble in water, ether, alcohol, and slightly soluble in cold and more soluble in hot petroleum ether. It has a sharp odor somewhat similar to that of butyric acid, although much milder. It readily reduces potassium permanganate in a cold alkaline solution. In a cold aqueous solution it decolorizes bromin instantaneously, but does not decolorize bromin in carbon tetrachlorid. With ferric chlorid it gives an orange color on the spot plate. In aqueous solution it does not reduce gold chlorid in the cold.

A determination of the neutralization equivalent (molecular weight) gave the following results: 45.3 mgm. of the soil compound required 10.43 c. c. of *N/10* sodium hydroxid (NaOH) for complete neutralization with phenolphthalein as the indicator.

The neutralization equivalent was found to be equal to 86.8.

The neutralization equivalent calculated for crotonic acid ( $\text{CH}_3\text{CH}:\text{CH}.\text{COOH}$ ) is 86.05.

The soil substance sublimes readily at room temperature, which is in accord with the observation made by Bulk (1).

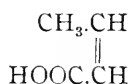
These reactions and tests on the soil compound and synthetic  $\alpha$ -crotonic acid were carried out simultaneously and were found to be identical in every case. The crystalline forms were also found to be the same. Figures 1 and 2 of Plate CXIV show the form and similarity of the crystals obtained in the first stage of sublimation. During the process of sublimation the crystals grow into large irregular plates or leaflets.

Ninety-four mgm. of the acid were obtained from 50 pounds of soil. This quantity would correspond approximately to 16 pounds per acre. It is obvious from the very unusual properties of this substance that a considerable amount would be lost in the processes of isolation and purification, and the actual amount present in the soil would be much greater than 16 pounds per acre, which is therefore a minimal value.

The  $\alpha$ - and  $\beta$ -crotonic acids are unsaturated and have the formula  $\text{CH}_3\text{CH}:\text{CH}.\text{COOH}$ . These acids are typical examples of compounds which exhibit geometrical isomerism. Their structures have been dwelt

<sup>1</sup> In all cases a slight softening or sintering at  $69^{\circ}$  to  $70^{\circ}$  was observed, which may be due to the presence of traces of  $\beta$ -crotonic acid. Morrell and Hanson (4, p. 1522) have shown that  $\alpha$ -crotonic acid, when heated above its melting point, is partially converted into  $\beta$ -crotonic acid in amounts varying with the temperature. This study indicated the advisability of subliming  $\alpha$ -crotonic acid at a low temperature in purifying it in our work. In order to prevent the loss of material by sublimation, the melting points were made in sealed tubes which were completely submerged.

upon by numerous investigators (7) and are represented by the following formulæ:

 $\alpha$ -crotonic acid $\beta$ -crotonic acid

Heretofore the occurrence in nature of crotonic acid has not been firmly established, and the formation in soils of a compound possessing such unusual chemical properties and structure is very difficult to explain. Schlippe (6) described an acid from croton oil which had the formula  $\text{C}_4\text{H}_6\text{O}_2$  and to which he gave the name "crotonic acid," but later investigations (2) on this oil have failed to show the presence of crotonic acid.  $\beta$ -Hydroxybutyric acid, which is present in diabetic urine, is readily converted into  $\alpha$ -crotonic acid by heating either alone or with dilute sulphuric acid (5).

$\alpha$ -Crotonic acid is also produced from allyl cyanid, which is a constituent of mustard oil. Krämer and Grodzki (3) have isolated crotonic and isocrotonic acids from pyroligneous acid obtained by the dry distillation of wood.

These methods of obtaining  $\alpha$ -crotonic acid suggest the possibility of its formation in soils during the destruction of cellulose, from  $\beta$ -hydroxy acids of the aliphatic series, or by the hydrolysis of allyl cyanid, which is found in the essential oils from certain plants.

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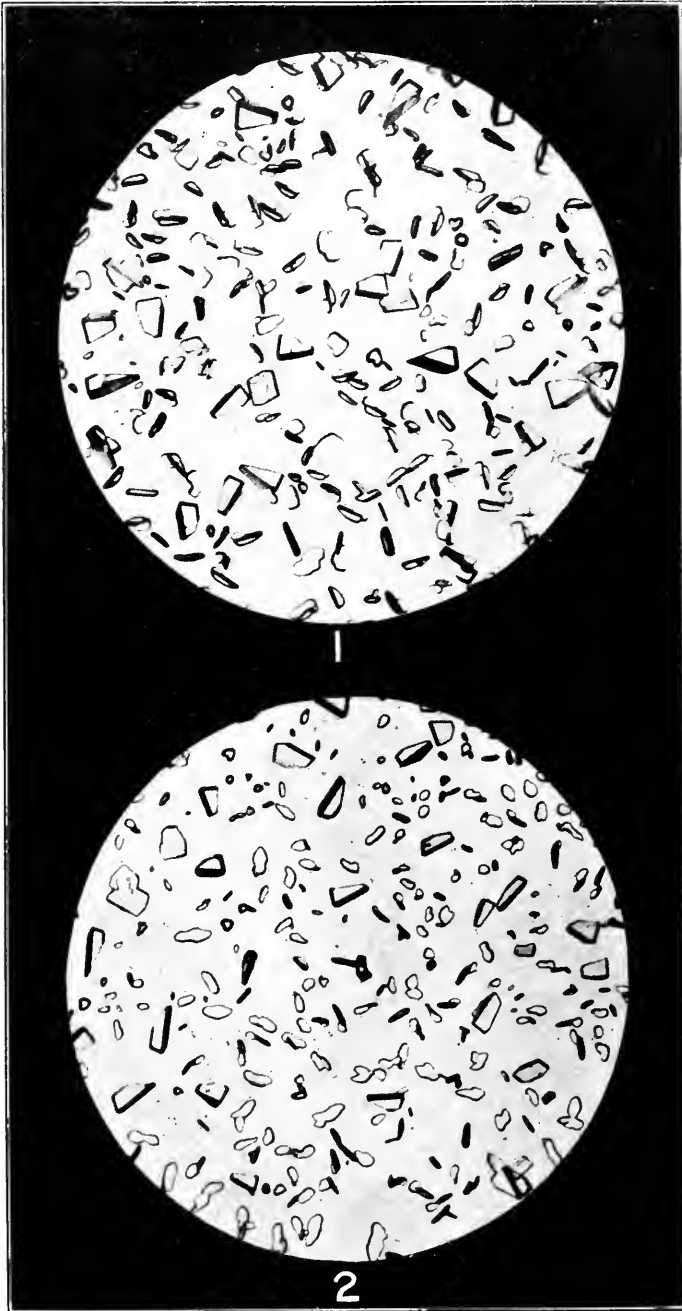
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PLATE CXIV

Fig. 1.—*a*-Crotonic acid from soil. × 210.

Fig. 2.—Synthetic *a*-crotonic acid. × 210.

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